Report on the Diseases of Silkworms in India

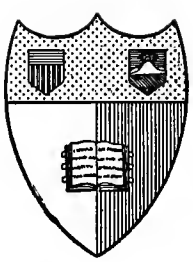
BY

A. PRINGLE JAMESON, D.Sc.

CALCUTTA
SUPERINTENDENT GOVERNMENT PRINTING, INDIA
1922

Price Rs. 3.

4/6



Report on the Diseases of Silkworms in India

BY

A. PRINGLE JAMESON, D.Sc.

CALCUTTA
SUPERINTENDENT GOVERNMENT PRINTING, INDIA
1922

Agents for the Sale of Books Published by the Superintendent of Government Printing, India, Calcutta.

IN EUROPE.

Constable & Co., 10, Orange Street, Leicester Square, London, W.C.

Kegan Paul, Trench, Trübner & Co., 68-74, Carter Lane, E. C., and 39, New Oxford Street, London, W.C.

Bernard Quaritch, 11, Grafton Street, New Bond Street, London, W.

P. S. King & Sons, 2 & 4, Great Smith Street, Westminster, London, S.W.

H. S. King & Co., 65, Cornhill, E.C., and 9, Pall Mall, London, W.

Grindlay & Co., 54, Parliament Street, London, S.W.

Luzac & Co., 46, Great Russell Street, London, W.C.

W. Thacker & Co., 2, Creed Lane, London, E.C.

T. Fisher Unwin, Ltd., 1, Adelphi Terrace, London, W.C.

Wheldon & Wesley, Ltd., 28, Essex Street, London, W.C.

B. H. Blackwell, 50 & 51, Broad Street, Oxford.

Deighton Bell & Co., Ltd., Cambridge.

Oliver and Boyd, Tweeddale Court, Edinburgh.

E. Ponsonby, Ltd., 116, Grafton Street, Dublin.

Ernest Leroux, 28, Rue Bonaparte, Paris.

Martinus Nijhoff, The Hague, Holland.

Otto Harrassowitz, Leipzig.

Friedländer and Sohn, Berlin.

IN INDIA AND CEYLON.

Thacker, Spink & Co., Calcutta and Simla.

Newman & Co., Calcutta.

R. Cambray & Co., Calcutta.

S. K. Lahiri & Co., Calcutta.

B. Banerjee & Co., Calcutta.

The Indian School Supply Depôt, 309, Bow Bazar Street, Calcutta, and 226, Nawabpur, Dacca.

Butterworth & Co. (India), Ltd., Calcutta.

Rai M. C. Sarcar Bahadur & Sons, 90/2-A, Harrison Road, Calcutta.

The Weldon Library, 57, Park Street, West, Calcutta.

Standard Literature Company, Limited, Calcutta.

Lal Chand & Sons, Calcutta.

Association Press, Calcutta.

Higginbotham & Co., Madras.

V. Kalyanarama Iyer & Co., Madras.

G. A. Natesan & Co., Madras.

S. Murthy & Co., Madras.

Thompson & Co., Madras.

Temple & Co., Madras.

P. R. Rama Iyer & Co., Madras.

Vas & Co., Madras.

E. M. Gopalakrishna Kone, Madura.

Thacker & Co., Ltd., Bombay.

D. B. Taraporevala, Sons & Co., Bombay.

Mrs. Radhabai Atmaram Sagoon, Bombay.

Sunder Pandurang, Bombay.

Gopal Narayan & Co., Bombay.

Ram Chandra Govind & Son, Kalbadevi, Bombay.

Proprietor, New Kitab-Khana, Poona.

The Standard Bookstall, Karachi.

Mangaldas Harkisandas, Surat.

Karsandas Narandas & Sons, Surat.

A. H. Wheeler & Co., Allahabad, Calcutta and Bombay.

N. B. Mathur, Supt., Nazir Kanun Hind Press, Allahabad.

Munshi Seeta Ram, Managing Proprietor, Indian Army Book Depôt, Juni, Cawnpore.

Rai Sahib M. Gulab Singh & Sons, Mufid-i-Am Press, Lahore, and Allahabad.

Rama Krishna & Sons, Lahore.

Supt., American Baptist Mission Press, Rangoon.

Proprietor, Rangoon Times Press, Rangoon.

Manager, the "Hitavada," Nagpur.

S. C. Talukdar, Proprietor, Students and Co., Cooch Behar.

A. M. & J. Ferguson, Ceylon.

Manager, Educational Book Depôts, Nagpur and Jubbulpore.*

Manager of the Imperial Book Depôt, 63, Chandney Chauk Street, Delhi.*

Manager, "The Agra Medical Hall and Co-operative Association, Ltd." (Successors to A. John & Co., Agra.)*

Supt., Basel Mission Book and Tract Depository, Mangalore.*

P. Varadachary & Co., Madras.*

Ram Dayal Agarwala, 184, Katra, Allahabad.*

D. C. Anand & Sons, Peshawar.*

Manager, Newal Kishore Press, Lucknow.*

Maung Lu Gale, Proprietor, Law Book Depôt, Mandalay.*

* Agents for the sale of Legislative Department publications only.

PREFACE

IN investigating the diseases of silkworms in India I have kept three things in mind—first the practical rather than the academic point of view ; second the historical standpoint, and third the results got in other countries, especially the tropics, as well as in India. Because of the first of these I have left out in this report all questions of a purely scientific interest. I hope to publish later in one of the scientific journals the results of my work on the life-history of the parasite that causes pebrine. Because of the second I have always discussed at some length the early work, especially that done in India, for only by paying due attention and respect to the older workers can one expect to get a true and complete view of any problem. Indeed, the work of the earlier investigators is very frequently on a much higher plane than our modern efforts : they were not so eager to get out results and so could spend more time in thinking over them. Because of the third I have not multiplied experiments. If I found that my results confirmed those of other workers I have not delayed further over them, believing that a fairly rapid survey of the field was more called for than a painstaking re-investigation of any particular point. I have written as simply as possible in order that my report might be intelligible to those who have no special knowledge of the subject : experts, if any should read it, will doubtless pardon any apparent " insults " to their knowledge.

I am deeply indebted to many people for assistance in carrying out this piece of work, but especially to the sericultural departments of the different provinces and States in India. From all I have received the utmost courtesy and assistance while touring in their districts, and they have shown the greatest kindness in replying to my inquiries. I have to express a special degree of indebtedness to Mr. P. C. Chaudhury, Deputy Director of Sericulture in Bengal, and to the staff of the Berhampore Central Nursery for having conducted experiments for me in the nursery and for having got rearers in the villages to rear worms for me specially. To all who sent cocoons for census purposes I wish to convey my thanks. For the photographs which illustrate several of my experiments I wish to thank Mr. Walton, Officiating Imperial Agricultural Bacteriologist, and for most of the drawings and text-figures I am indebted to Mr. K. D. Das and Mr. N. N. Bagchi, two of the artists on the Pusa staff. Finally, I would record my appreciation for the way in which my assistants, Mr. Harihar Prasad and Mr. C. B. Sahaya, have helped me in my work, and I would also name my head rearer, Lotan.

TABLE OF CONTENTS

A Report on the Diseases of Silkworms in India.

I. INTRODUCTION.

HISTORICAL SKETCH OF DISEASE IN INDIA.

The question of silkworm diseases is no new one in India, although it is only in comparatively recent times that any really reliable data are to be found regarding this subject. Despite the fact that sericulture is probably a much older industry in India than in Europe, there are no records, so far as I am aware, which show what the condition of the worms was in the early centuries—nothing comparable with the treaties on sericulture by Olivier de Serres published in Europe in 1599. There are references in Indian literature to sericulture,* but in these no account of the methods and trials of the rearer are given. It was not until the arrival of the traders from Europe in the 17th century that attention began to be paid to means whereby the increase of silk could be attained.

The early reports of the East India Company contain many references to the trade in silk, and some of them seem to me to indicate that it is at least possible that disease was not absent, although there are no direct references to any epidemic. The repeated attempts made to introduce foreign stock—Chinese in 1710 and 1770, Italian in 1795 and 1833— might indicate that all was not well with the local races, or it might merely be the desire to try to obtain a worm yielding more and better silk. There are, however, several references to the degeneracy of the worms which make one think that diseases must have been prevalent although they were not specifically mentioned. Thus Italian worms introduced in 1795 all "perished"; while in 1796 a report by the Resident of Jungypore—"an authority on silk"—deals with the question of degeneracy and puts it down to three causes: 1 "improper food";

* My first assistant, Mr. Harihar Prasad, has called my attention to some lines in the Ramayan of Tulshi Das, written about 1574, Chapter 7, Part VI.

"Pát keet te hoi, táte pátamber ruchir,
"Krimi pálc sab koi, pram apáwan prán sam."

He has translated them for me freely as follows:—"Silk is produced by an insect, from that beautiful silk-cloth is prepared. Although the insect is most untouchable, many people care for it as carefully as their very lives."

Evidently as far back as the 16th century sericulture was a well known industry in the plains of India.

2 "improper management of the worms"; 3 "the present mode of conducting the Company's silk investments." Again in 1819 the Resident of Jungypore complained that the cultivation of the "Bara Palu" worm had become "extremely p ecarious and uncertain." It is highly probable that such facts as I have mentioned point to a considerable amount of disease in Bengal.

The Company attempted to deal with the question of degeneracy chiefly by calling for reports from its servants—a method not unknown to this day—but also, as we have noted, by introducing foreign stock and fresh varieties of mulberry, although, as Geoghegan remarks, these efforts "were not so vigorous." It is interesting to note that the "carelessness and improper management of the natives" as well as their tendency to starve the worms are cited as being among the chief causes of "degeneration."

After 1834 the Company ceased to take an active part in the production of silk, which was left in the hands of private individuals, so that the Company's records no longer continue to be of any value to us. A considerable amount of work on the improvement of sericulture was done by various people, sometimes amateurs, sometimes professional sericulturalists; at times encouraged by Government, at other times ignored. And from the reports and papers of these workers one gathers that there was a considerable amount of disease to be found, when the work of rearing worms was personally superintended by an observant and intelligent person of education.

A certain Mr. Bashford, a filature manager, experimenting about the years 1854–56 on the hybridizing of silkworms had very varying success with his worms. At times "many thousands of the worms died," or again they had "very fair success with their worms, which escaped the disease then prevalent among native worms." But finally disaster seems to have overtaken his rearings—"the crop this time was a great failure, the worms deteriorated—and cocoons were light, flimsy and a perfect disappointment." Mr. Bashford concluded from his experiments that "more careful cultivation of the mulberry; a fuller supply of leaf to the worms; more attention to selection in breeding, to ventilation and equable temperature" might be expected to improve the silkworm.

About this time a great deal of work was done by Captain Hutton, and in a series of papers from 1859 onwards he records his observations. There is no doubt about the disease in silkworms in these publications. Although the author was evidently not in a position to diagnose the maladies accurately—who was prior to Pasteur's work of 1865–1870?—it is obvious that pebrine and grasserie at least were serious plagues. The suggested remedies are

more care in growing the mulberry, more care in feeding the worms—especially feeding during the night and feeding always on freshly gathered leaf—better ventilation and better spacing of the worms.

It is impossible to note all the records of disease and remedies for improving the silkworms, so that I am selecting only a few of the more outstanding cases. Thus in 1867 an attempt to introduce sericulture in Bihar resulted in disease sweeping off all the rearings. While in Mysore in 1866 a disease broke out which was described as "a sort of atrophy" and was doubtless an epidemic of pebrine. In every case in which work on sericulture was conducted by Europeans attention was drawn to the fact that the native rearer was neither very careful nor cleanly in his methods, and that if sericultural practice were improved the question of disease would tend to disappear. Strangely enough, however, when this advice was put into practice for a little while under European supervision disease did not disappear as was expected.

While private individuals were doing most of the work of value, the various Governments in India continued to make more or less spasmodic attempts to do something for sericulture. In Bengal little seemed to have been done but in Bombay, Madras, Mysore, Punjab, North-Western Provinces and Kashmir the various Governments seem to have in various ways encouraged sericulture but at this particular period with but little success.

During the years with which we have been dealing in the last three paragraphs—that is to say, from about 1850 to 1870—disease had been rampant among the silkworms of Europe, and sericulture had been almost killed out in France and Italy. Many investigations were conducted into the question of silkworm diseases, and finally the famous French bacteriologist, Pasteur, completed his masterly researches, and by his papers and his book on "La Maladie des Vers à Soie," published in 1870, brought comos out of the chaos in which the subject was weltering. The great falling off in the silk production in Europe turned the silk merchants' eyes once more to the East, and the Government in India began to take a more active interest in sericulture. The only immediate result was the very able report by Geoghegan on "Silk in India" published in 1872. To this work I am indebted for many facts quoted above. But there was now some definite knowledge available on the subject of disease, and some improvement was to be expected—if not in the worms themselves at least in the accounts published of their condition.

In 1886 and again in 1887 a conference was held in Calcutta on sericulture, and Wood Mason and N. G. Mukerji were asked to investigate the problems of disease in silkworms. The result of

their investigations was the discovery that " in Bengal, mulberry silkworms suffer from diseases which have been found to be identical with the diseases known in Europe." Mukerji was then sent to Europe in 1888 to study sericulture there, and on his return to India the Government started nurseries in Bengal for the production of disease-free seed.

Mukerji was keenly interested in sericulture and published a manual on sericulture in Bengali and his well-known " Handbook of Sericulture." These works are now naturally rather out of date and in some respects are not very scientific, but the latter contains much interesting and useful information. Unfortunately, Mukerji advocated what he called a modification of Pasteur's method of seed selection which, as we shall see later, was of very little use. For this reason he has been rather fiercely criticised, but it should be remembered that he was doing pioneer work with really very little encouragement, and that actually, despite all discouragements and opposition, he did effect some improvement in sericultural methods. I have come across rearers who proudly claim to be Mukerji's men or the sons of men who learned from Mukerji, and they are all rearers distinctly above the ordinary level of native rearers. The Calcutta Silk Committee recorded that " with the eradication of disease considerable improvement in the yielding capabilities of the cocoons has already been obtained." But his nurseries seem to have failed in their purpose on the whole—due to the fact that, as I have said above, his method of seed examination was bad.

No exact investigations of disease had been made, and it seems to have been taken for granted that disease was very serious, but that the establishment of Government nurseries would set matters right. All this time there was much controversy over the subject of sericulture as a cottage industry. Since about 1880 a certain Mr. Lister had been attempting to grow silk on a large scale, first in the Punjab and later at Dehra Dun. After some initial failures he succeeded well, according to his own showing, and in 1889 wrote that " this great problem is now completely mastered and that the future of sericulture in India is now assured." Unfortunately by 1892 his ventures had to be abandoned, evidently on account of losses caused by disease. Mr. Lister's contention was that the native rearer was the cause of most of the " disease." " Disease," he wrote, " about which we hear so much, is only another name for ignorance, neglect, dirt and rearing-houses altogether unsuited for the purpose." Despite the fact that his undertaking failed, Lister's " three or four simple rules " for sericulture are very sound and may be quoted here: " First, sound seed; second, air, space and cleanliness; third, regular feeding; fourth, suitable rearing-houses." The

mistake made was in doing things on too big a scale. Mukerji was right. Sericulture is a cottage industry, and disease can be kept in check only when relatively small numbers of worms are kept together.

With such experiments and controversies the regime of N. G. Mukerji in Bengal sericulture was concerned.

In passing, another name, that of Cleghorn, which is honourably associated with Bengal sericulture, may be mentioned. He paid much attention to hybridization and selection, but to him we are indebted for the fullest account of the Bengal silk fly. Unfortunately he does not seem to have been in favour of microscopic selection of seed, so that the weight of his influence was not thrown always into the right scale.

But sericulture continued to languish. Another government committee was appointed in 1908, and a number of recommendations were made and not—as a rule—carried out. The work of the seed supply stations went on but the most important work seems to have been that of hybridization, which had been repeatedly tried in the past and had failed, and which up to the present has not yielded—as far as I can see—very encouraging results.

In other parts of India during the period from 1890 to about 1914 very little was done on a large scale—except in Kashmir where a flourishing industry was established, and where disease has been more or less got under control. In Mysore the silk industry is comparatively modern, having been introduced about 1780 by Tippu Sultan: Lefroy (1916) calls this an old industry but compared with other regions it cannot be considered as other than young. After the English took possession of this State, serious attempts were made to encourage sericulture, but by 1870 they had nearly all been abandoned on account of failures due evidently to disease. The industry remained in a very decaying condition until the second decade of the present century when the Government took it up, and with the help of Italian and Japanese experts got a thoroughly satisfactory Department of Sericulture started. Under the energetic and wise management of the present Superintendent of Sericulture, the department is doing excellent work in all branches of sericulture and diseases are being very efficiently controlled. In other parts of India sericulture is not an industry of any size with perhaps the exception of Assam. In this province, however, it is only within the last year or two that any attempt has been made to organize sericulture.

Much of the recent advance in sericulture in India is doubtless due to economic reasons, but possibly Lefroy's enquiry into Indian sericulture in 1915 has had something to do with it. In this the

latest and most detailed Government contribution to the study of sericulture in India, much stress is laid upon disease as a factor in the decline of the silk industry in this country, and many recommendations are made on every subject connected with sericulture. Acting on Professor Lefroy's suggestion, a beginning was made in 1916 by the Imperial Agricultural Bacteriologist in the study of disease in silkworms. In 1919 I was sent out specially to do this work, and the following report is an account of my findings in this field.

Such is, very shortly and very imperfectly, a slight history of what we know of silkworm diseases in the past in India, and what steps have been taken to deal with the problem. It is extremely difficult to generalize on " silkworm diseases" in India, because in this country not only are there at least five quite distinct diseases to be reckoned with, but there are three different genera of silkworms concerned. I propose, therefore, to deal with each disease and each silkworm separately, making such deductions and such recommendations as seem called for under each section, and finally giving a summary of general conclusions and recommendations.

II. DISEASES OF MULBERRY SILKWORMS.

Introduction. The term " silkworm," while in reality a vague one, has practically come to mean the mulberry silkworm, the caterpillar of various varieties or species of the genus of moths, *Bombyx*. There are of course silk-producing caterpillars of other genera, such as the Eri (*Attacus ricini*), the Muga (*Antheræa assamensis*) and the Tasar (*Antheræa mylitta*), but the industries associated with these worms in India are so small that they are but little known to the world at large, and even in the silk industry they take a very unimportant place. However, as the subject of our enquiry is " silkworm " diseases, they will have to be considered ; but I propose first to discuss the diseases of mulberry worms, and having investigated these in some detail, it will be found that the diseases of the less important producers of silk may be disposed of rapidly.

There are several different species or varieties of mulberry silkworms, but it would be quite outside the scope of our investigations to enter into an essay on these and their validity. It will be sufficient for our purpose to note that, excepting in Kashmir where a univoltine (*i.e.*, producing only one generation in a year) worm of French or Italian origin is reared, the great majority of the worms reared in India are multivoltine (*i.e.*, producing many generations in a year). There are several different kinds of multivoltine worms reared in different parts of India, such as the Nistari and Chhotapolu of Bengal, yielding a golden yellow cocoon, the Mysore, giving a greenish white cocoon, and the Burmese, spinning a large loose textured cocoon, but all appear to be on the whole subject in the same degree to the same diseases, so that it will be unnecessary to treat them separately.

The mulberry silkworm is a completely domesticated animal which has been reared by man for at least 4,500 years, and like all domesticated animals—and indeed like wild animals too for that matter though in a much less degree—it is subject to certain diseases and pests. Our knowledge of silkworm diseases is, however, of comparatively recent date. Despite the fact that silk and the rearing of silkworms are referred to in Chinese records said to date back to more than 2,000 years before the Christian era, the earliest definite records of disease are to be found in the first European treatise on sericulture, that by Olivier de Serres published in 1599. Here the susceptibility of worms to disease is clearly recognized and

an endeavour made to describe and define the diseases. In this, as indeed in many of the much later works on sericulture, the symptoms of several diseases were confounded together, and, as was to be expected, only the more striking diseases and their most characteristic appearances, such as the white efflorescence on the mummified bodies of worms that had died of muscardine, were noted. The significant point is, however, that at this early date in the history of sericulture in Europe disease was well established among the worms and evidently not a thing of recent years. Subsequent works on sericulture continued to pay attention to the question of disease in European silkworms, and gradually many different forms of sickness were recognized, some of them merely being names for what are now recognized as different symptoms of the same disease. It was not until the work of Pasteur in the years 1865–1870 that order began to appear in the diagnosis and treatment of silkworm diseases. Since his day much work has been done on this subject, and a series of more or less well marked diseases are now recognized.

The diseases of silkworms may be divided into two classes : those caused by certain easily recognized animal and plant parasites, not bacteria, and those of a more indefinite nature in which bacteria may or may not play a part. For want of better names I shall call the first group "parasitic diseases" and the second "rot diseases." Under parasitic diseases I include three diseases :— (1) Pebrine. caused by *Nosema bombycis*, (2) Muscardine, caused by *Botrytis bassiana*, and (3) the Fly pest, caused by *Trigcolyga bombycis*. Under rot diseases I include all diseases of the flacherie type and grasserie. The parasitic diseases are reasonably well understood and their diagnosis and control are consequently possible. The rot diseases, on the other hand, are somewhat obscure in their origin and consequently imperfectly understood and difficult to control. All these diseases are not equally important in India. I propose to take them in turn devoting space to them in proportion to their importance. When all the diseases have been examined, we shall turn our attention to the other silkworms found in India and rapidly run through the diseases from which they suffer.

1. KATA OR MATHA-KATA (PEBRINE).

Definition and diagnosis. The name pebrine is in reality an unfortunate one for this disease. It was given to it because of the prevalence of black spots on the skin. According to De Quatrefages, who gave the name currency, pebrine in the language of the Midi means "pepper disease"—the black spots resembling pepper

sprinkled over the skin of caterpillars affected by it. But in reality this is by no means one of the most characteristic features of the disease, at least in India. Some of the older names such as "maladies des petits" or "atrophie," signifying the uneven growth of the worms, would have been much more suitable, but the name is now much too well established to be changed. The Bengali name Kata or Matha-Kata is said by Mukerji (1899) to refer to the pale colour that is said to be characteristic of pebrinised worms in India.

Worms suffering from pebrine do not show any symptoms visible to the naked eye until the disease is far advanced. Then it is seen that the worms become more and more unequal in size—some growing normally while others remain very small. The worms are sluggish and slow and irregular in passing their moults, and are said to be paler or more translucent than healthy ones. There may be considerable mortality among them, and then it will be found that the dead worms do not become at once rotten and soft but tend to be dry and rather firm. In some cases the black spots referred to above are found, but in India these are not very common. The spots are irregular in form and very dark brown to black in colour. They are to be found all over the body (Plate I, figs. 2 and 5). The most characteristic feature of this disease, however, is the presence, in different parts of the diseased caterpillar's body, but especially in its gut, of numerous minute oval bodies, which are the spores of the parasite which causes the disease. A full account of these and the life-history of the parasite will be given below. Should caterpillars affected with this disease spin cocoons—and the majority frequently do so—the cocoons will be flimsy and poor, and the moths which cut out of them may be deformed (Plate I, fig. 3) with small, often scorched looking, wings and distorted antennæ, and what is much more serious, the eggs laid by the moths are laid irregularly, many do not hatch and of those that do many may give rise to diseased caterpillars.

History of the disease. It is, I think, very important that the history of this disease should be thoroughly well known, for there seems to be an idea in India that it is a recent thing, at all events in this country. Thus in Geoghegan's "Some account of Silk in India," 1880 Ed., it is stated that "no epizoic such as the 'muscardine' and the 'pebrine,' which have devastated France and Italy, has as yet appeared in India." N. G. Mukerji reported in January 1888 "that while flacherie, grasserie and muscardine have always been known in Bengal (note that Geoghegan evidently had not heard of muscardine in India, showing how reliable these statements are) pebrine has only appeared within the last ten or

twelve years, becoming each year more destructive and causing fears of a total collapse of the trade." Finally, in the section on pebrine in Lefroy's "Report on an inquiry into the silk industry in India" it is actually stated that "this disease appeared in Bengal about 1895 and probably in Mysore between 1890 and 1900." The date 1895 is obviously a misprint for 1875 but what of the statement regarding Mysore? Later in the same section Mr. Hutchinson writes, "in all probability the comparatively small part which pebrine has played in depressing the successful rearing of silkworms in India up to the present time is merely due to the comparatively short time which has elapsed since the introduction of infection and that, in the absence of effective measures for dealing with it, its future spread and expansion to dimensions similar to those attained in France in the middle of last century is only a matter of time"—a clear expression of the opinion that pebrine in India is a very new disease. In the light of such views it is necessary to try to determine if pebrine is really a thing of recent introduction into India.

The fact that pebrine was not recognized as a definite disease in Bengal prior to about 1875 is not to be wondered at, for it was not really properly recognized anywhere until the researches of Pasteur (1865–1870) gave a certain method of diagnosis—that is to say, the examination under the microscope of a portion of the body of the moth selected for seed production and the recognition of the presence or absence of the spores of *Nosema bombycis*. It is true that the spores had been seen some fifteen years earlier, and that Italian workers had paid some little attention to them in connection with their investigations into silkworm diseases, but it was Pasteur who demonstrated conclusively the connection between pebrine and the "corpuscles"—that is to say, the parasitic nature of the "corpuscles"—and showed how these could be used as a means of diagnosing the disease. But although it was not until about 1870 that the technique for diagnosing pebrine with certainty was evolved, there are numerous indications in the earlier writings on sericulture that this disease was no new plague. In the treatise on sericulture by Olivier de Serres, to which I have already referred, and which was published in 1599, there is very good evidence that a disease characterized in part by the presence of spots on the skin was in existence in those days. It was confused with grasserie, it is true, but as pebrine is the only known disease of silkworms that characteristically shows this spotting of the skin, it is practically certain that we are here dealing with this disease as well as with grasserie. Later works, such as those of Boissier de Sauvages (1763) and Dandolo (1818), give descriptions of various diseases in which spots on the skin are cited as symptoms. It may therefore be fairly

safely assumed that pebrine has been in existence in Europe for over three hundred years. Indeed, Pasteur was inclined to the belief that certain serious epidemics which in the end of the 17th century, in 1750 and again in 1780 threatened to exterminate the silkworm in France had been outbreaks of pebrine. Now if pebrine has been a recognized disease of silkworms ever since their introduction into Europe or at all events ever since men of intelligence took a sufficient interest in sericulture to write treatises on it, is it likely that the East—the home of the silkworm—should be free from this disease ? To my mind the matter admits of no doubt: pebrine is no more a new disease in the East than it is in the West. The fact that we have no early records of it in the East is not to be wondered at, for there are no writings of any such description on sericulture in India. The work was and is in the hands of illiterate peasants, and the mind of the educated and intelligent inhabitant of the country does not at present and doubtless did not in the past run, as a rule, to biological investigations.

But even for the Far East we have direct evidence that pebrine was in existence at least ten years earlier than 1875. Pasteur showed that seed got from Japan in 1865 was infected with the disease. So the Far East was not really free from pebrine, although Japan had had that reputation: it required only a careful examination of their stock to find it tainted. The worms of the Near and Middle East had by that date shown themselves to be highly diseased. Is it likely that India out of all countries in the world should be free ? The idea is preposterous.

But indeed actual evidence of the presence of pebrine in India prior to 1875 is not wanting. In a paper by Captain Hutton dated November 1859 (*See* Geoghegan, 1880, page 113), the description given of diseased "Boro poloo" eggs is obviously that of a pebrinised laying. On the same page, too, mention is made of the "black spots appearing at the junction of the annulations of the body." A disease in Mysore which was described as "a sort of atrophy" in 1866 was also doubtlessly pebrine.

Significant, if indirect, evidence of the comparative antiquity of pebrine is found in the history of the causal organism. As will be seen below, this organism, *Nosema bombycis*, is a member of an order which characteristically parasitizes insects. As far back as 1851, when the group first began to be studied, all sorts of insects, including several caterpillars, were found to be infected with the same or very nearly related parasites. Here then we have a well defined group of parasites commonly found in insects, and it is actually claimed that the fact that it was not noticed in silkworms in India prior to 1875 proves that it has been "introduced" in the country

at about this time. To the credit of N. G. Mukerji be it noted that he did not believe pebrine to be a recent introduction, for he says "that the plague has spread throughout the world during the last 50 years does not seem to be due to the spread of the germs connected with it from one country to another." He evidently recognized that the germ of the disease was in India all the time, but thought that only about 1875 it began to cause disease. He blames domestication for rendering the silkworm more susceptible to the disease, but as silkworms were domesticated in the East many centuries ago I should have thought that the disease might possibly have made its appearance prior to 1875, if it is admitted that the causal organism was in the worms all the time.

I believe we are justified in stating the matter in this way. Many insects are infected with a parasite belonging to the order Microsporidia. Like the huge majority of parasites these organisms do not damage their hosts under ordinary conditions. If, however, conditions are changed these parasites are liable to cause injury to the insect attacked. In the wild state or under natural and normal conditions there is a balance maintained between all living organisms, and so between host and parasite. Upset the ordinary conditions in any way, and in every likelihood that delicately adjusted balance will be destroyed and disaster will result, usually to the host. Silkworms, in the wild state, like so many other insects, harboured a parasite, *Nosema bombycis*. When they were domesticated they brought their parasite with them into men's houses. There the conditions were somewhat different from out in the wild, though probably at first the worms were reared under very much more natural conditions than they are at present. Thus by domestication their balance of life was interfered with. Doubtless the worm became less strong, less resistant: the conditions under which they lived tended to make the infection of the worm by the parasite easier. In this way a normal harmless parasite became a disease-causing parasite. As domestication was probably gradual—indeed in China to-day silkworms are sometimes reared in the open—no very serious results of the disease were noted as long as rearing was done on a very small scale. As soon as larger rearings were attempted—as soon as rearers in any way tried to force the worms—then disaster followed.

The only thoroughly studied epidemic of pebrine—that in Europe between 1850 and 1870—shows clearly that the most probable cause of the plague was the large rearings which were attempted about 1850. Thus while the average French crop in the years 1841–45 was 17,000,000 kilogrammes, in the following five years it rose to 21,000,000 and in 1853 it was actually 26,000,000. In attempting

to rear half as many worms again as 10 years previously, without any proportional increase of accommodation, the industry was overtaken with disaster. The parasite, always present in some worms, increased to an appalling extent, destroying the worms wholesale, and the industry came near to perishing. This is the only detailed account, as I have said, of a pebrine epidemic, but it is interesting to note that Lefroy (1916) in his report draws attention to the fact that " pebrine is supposed to have become serious about the time (1875) that prices were falling heavily ": when this occurred " the rearer strives to cut down expense, and to increase his brood." " I think we may assume that the rearer, in the years of falling prices, probably brought out the pebrine epidemic by bad feeding and directly brought on the condition that ruled for some years." That, I believe, is a true statement of the case. Pebrine is nothing new, but under ordinary conditions the parasite does not assert itself so that its presence is overlooked. As soon as the rearer deviates in the slightest from the best practice of rearing, the parasite goes ahead, and an outbreak of pebrine is the result.

I have somewhat insisted on this point because it seems to me that in India there has been a tendency to look upon pebrine as something new, and as such something that may at any moment assume very serious proportions. This view appears to me quite wrong. The disease is not a new one, and under the present Indian conditions it is not likely to become any worse than it has been during the past forty-six years—the period fixed by Mukerji since its assumed appearance in Bengal—or during the unknown and unnumbered years that have passed since its actual origin. There is no call for alarmist statements. The parasite of the disease is present in India and probably has been for a very long time. In the past it has probably—almost certainly about 1875—assumed epidemic form when economic conditions were such as to induce the rearers to depart from their customary practices, and in the future it is likely to behave in the same way. As we shall see later, they have a worm to rear that is very hardy and is accustomed to the minimum amount of food. They cannot well under-feed or overcrowd more than they do at present, so that on the whole it is probable that things will remain much as they are. Rearing conditions will stay the same, so that the balance which has been established between parasite and host will not be greatly disturbed, and we are not likely therefore to have any serious outbreak: it is not to be expected that " its future spread and expansion to dimensions similar to those attained in France in the middle of last century is only a matter of time." While wishing to stop once and for all exaggerated ideas about pebrine, I do not intend to minimize the

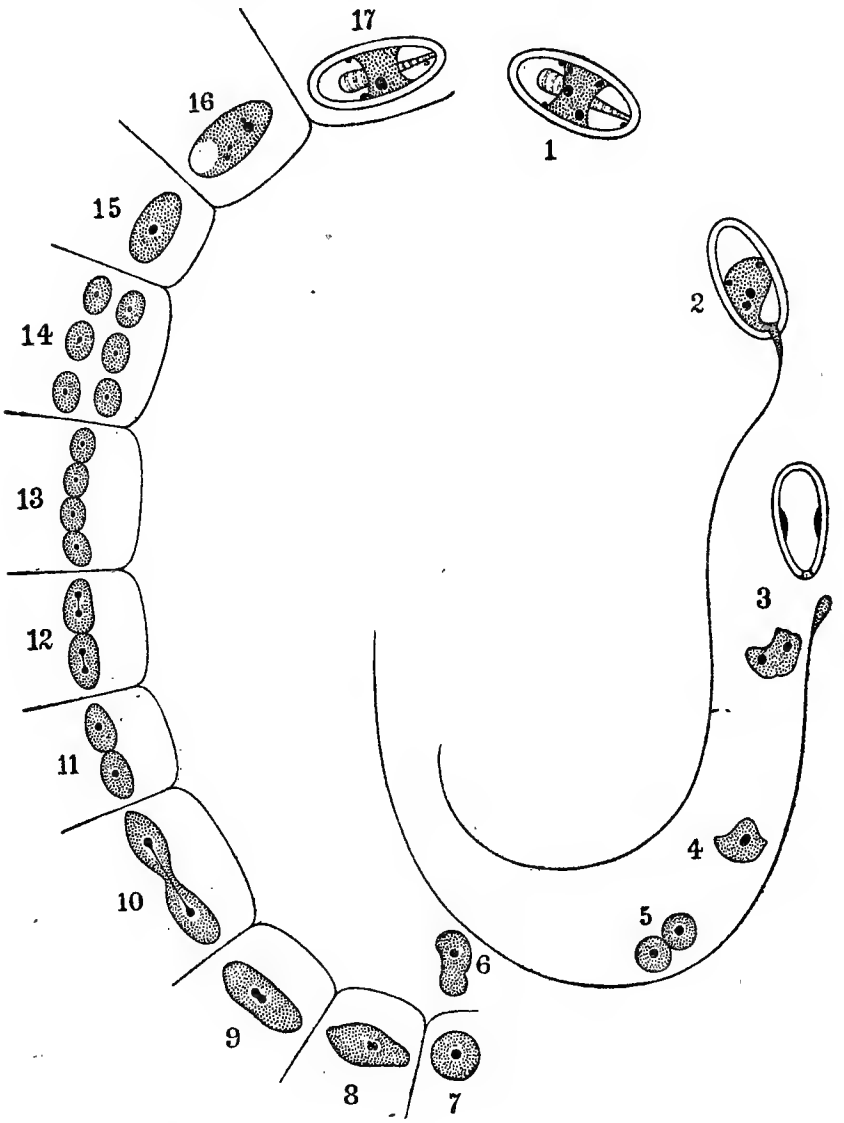
seriousness of the disease. Where no measures are taken to cope with it, it causes great loss and it is undoubtedly a serious menace to the success of sericulture in this country; but it is this constant and real lessening of the silk yield through pebrine that is to be feared and guarded against rather than some problematical epidemic of the future.

History and life-history of the causal organism. The disease pebrine is caused by a protozoan parasite belonging to the order Microsporidia of the subclass Neosporidia of the class Sporozoa. The large class of the Sporozoa are without exception parasites, and the order Microsporidia are pre-eminently parasites of Arthropods, that is to say of Insects and Crustacea. The organisms belonging to this order were first discovered during the investigations into the terrible epidemic of pebrine that swept through the silk-rearing districts of Europe and the Near East between 1850 and 1870. It is to Leydig and Balbiani that we owe the first really scientific investigations of this group. These workers discovered parasites of this order not only in silkworms, but in other caterpillars, in beetles, in crane flies, in bees, in scale insects, in spiders and in water-fleas (a small crustacean). More recently, members of this same order have been described from the river crayfish and from some fish such as the flounder and the stickleback, as well as from many other insects than those named above. The Microsporidia are thus not by any means a very small group of parasites, nor are they of very recent discovery. They are characterized by the production of minute oval spores of a somewhat peculiar construction. In the genus *Nosema*, to which the pebrine organism belongs, each organism produces only one spore, the other genera in the order producing more than one spore per individual. Before proceeding to the description of *Nosema bombycis* and its life-history, it may be mentioned that another species in the same genus, *Nosema apis*, a well known parasite of bees, was at one time supposed to cause the Isle of Wight bee disease, but is now known not to be responsible for this terrible pest causing only a mild form of sickness—*Nosema* disease—with very low mortality (White, 1919). It is an interesting point, however, that the only two insects really domesticated by men should be infected by such closely related parasites.

In describing the life-history of *Nosema bombycis* I do not propose to enter into any scientific details regarding its development. I want merely to give a general outline which may be of use and of interest to those engaged in sericulture in India. For details see Stempell (1909) and Kudo (1916).

The spore, which has been mentioned above, is the most suitable

point in the life-history at which to start our description, as it is by means of the spore that infection is spread in the ordinary way.



Text-figure A. Diagram of the life-history of *Nosema bombycis*. Modified somewhat from Stempell and Minchin.

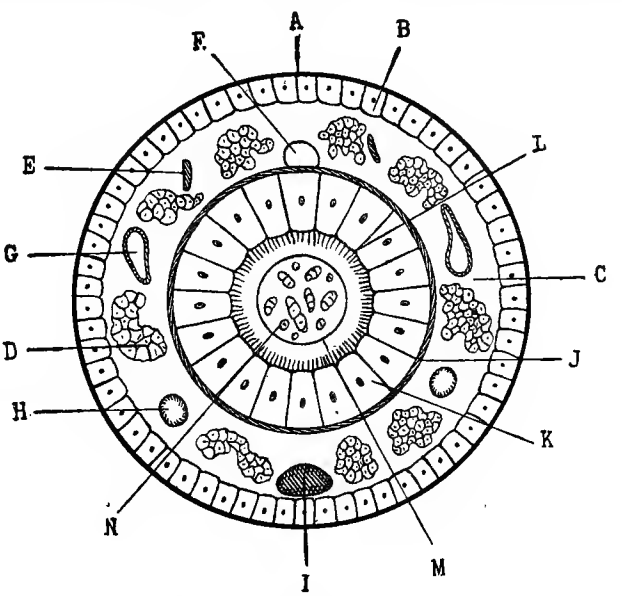
(1) Ripe spore with girdle of living substance with nuclei, two vacuoles and polar capsule (2) Polar filament extruded; planont about to leave spore-case. (3) Planont with empty spore-case and polar filament immediately after leaving spore-case. (4) Planont with one nucleus. (5) Fusion of two planonts or division. (6) Planont about to enter gut-wall cell. (7) Meront in gut-wall cell. (8)—(11) Division of meront into two within gut-wall cell. (12)—(14) Further divisions of meront. (15) Meront ready to form spore. (16) First stage in spore formation—one main and three subsidiary nuclei and one vacuole formed. (17) Further stage in spore formation—thick spore-case secreted, two vacuoles and polar capsule.

The spore is a very minute oval body measuring as a rule 3-4μ * in length and 1·5-2μ in breadth. Much larger spores than this are occasionally got, some having been measured as much as from 6 to 10μ long. When seen in the fresh state, that is untreated by reagents, little or no internal structure is visible. It appears as a very bright oval body standing out as a rule clearly from its surroundings. (Plate VIII, fig. 5.) It is this appearance, due to its high refractive index, that makes it possible for the spores to be recognized under comparatively low powers of the microscope. The "line" which is described (*See* Mukerji, 1899) is of course merely an optical effect. When treated in a suitable fashion, however, and coloured by certain stains, the contents of the spore are revealed. The internal structure of the spore varies somewhat according to its stage of development, but taking a nearly fully developed individual it presents an appearance such as is shown in the Text-figure A, 1. The spore wall is seen to be thick. At each end is a clear space or vacuole, and in the middle is a coloured portion surrounding the inside of the spore like a girdle. This of course refers to a killed and stained specimen : in the fresh spore this girdle is colourless. This is the part that in a living spore would be destined to infect a fresh caterpillar. Contained in this stained portion are two darker, roughly circular particles, the nuclei : other nuclei may at certain stages be seen, but as these have no very evident connection with the future history of the organism, they may be neglected. Very occasionally a faint, pear-shaped sac may be made out towards the middle line and one end of the spore. But this is at best but a fleeting impression. If, however, the fresh spore is treated by certain liquids, a very long thread is shot out from this sac-like body (Text-fig. A, 2; and Plate III, fig. 4). The easiest way to demonstrate this is to treat some fresh spores with hydrogen peroxide when a vigorous shooting out of these polar filaments, as they are called, will be seen (*See* Kudo, 1918). The use of these filaments seems to be this : when spores of *Nosema bombycis* are eaten by a silkworm, or if one directly feeds a caterpillar on spores for experimental purposes, the juices in the gut act upon the spores and if they are ripe, or fully formed, in a very short time after they arrive in the gut the polar filaments are shot out, and seem to anchor the spore to the gut wall. It is supposed that the digestive juice of the caterpillar acts on the spore in the way that hydrogen peroxide does and stimulates it to shoot out the filament. The peculiar thing, however, is that if one treats spores with the digestive juice of a silkworm outside the caterpillar's gut, say, on a microscope slide,

* The "μ" used in microscopic measurements is employed here on account of the convenience in writing it. It is one-thousandth part of a millimeter or roughly one-twenty-five thousandth part of an inch.

the polar filaments are not shot out. Some workers claim to have secured emergence of the filament by treating spores in this way with digestive juice, but I have not found it possible under ordinary experimental conditions.

Suppose now that a silkworm has by some chance eaten leaf contaminated with spores and these spores have reached the gut of the worm and shot out their polar filaments. The next thing to happen is for the contents of the spores to creep out of the spore cases. We have already noted the spore contents in the stained specimen as forming a ring round the inside of the spore case and containing two nuclei. When this little mass of living matter makes its way out of the spore, through the hole through which the polar filament shot, it is seen as an exceedingly minute, somewhat irregularly shaped body about 2μ in diameter if it were measured in a somewhat rounded up condition (Text-fig. A, 3-6; and Plate III, figs. 5 and 6). Of course such a tiny organism as this cannot be studied except under high powers of the microscope and after having been specially stained so that one can distinguish it from its surroundings. But even with the greatest care it is only with difficulty that the behaviour and structure of this body can be observed, and therefore our knowledge of it is not nearly so complete

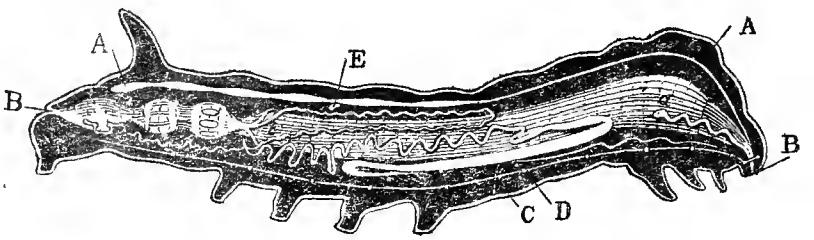


Text-figure B. Diagram of cross-section of caterpillar.

A, Ectoderm or outer skin. B, Hypodermis or under skin. C, Hæmocœle—the space, filled with blood, in which the various organs lie. D, Fat body. E, Muscle. F, Dorsal vessel or heart. G, Silk-gland. H, Excretory tube. I, Ventral nerve cord. J, Basal membrane of gut. K, Epithelial cell of gut. L, Fibrillar fringe of gut cells. M, Peritrophic membrane. N, Lumen of gut with leaf particles.

and accurate as could be wished. This little organism is called a "planont."

The planont moves by a peculiar creeping mode of locomotion called amœboid. It passes through the peritrophic membrane (Text-fig. B), assisted most probably by the production of some dissolving or digesting substance, and finally penetrates the gut-



Text-figure C. Diagram of dissection of caterpillar (From Mailott et Lambert).

A-A, Dorsal vessel or heart. B-B, Gut. C, Silk-gland. D, Nerve cord. E, Reproductive organ. *a*, Salivary gland. *b*, Excretory tube. Hæmocœle is black. Note junction of fore and mid gut just in front of "a" and of mid and hind just behind "b".

wall proper. It seems almost certain that at this stage in the infection the planont in its journey gets no further than the gut-wall: the firm muscular basal membrane to the outside of the gut seems to defy further progress.* At all events if the guts of infected silkworms are examined during the first few days after infection, the parasites are practically always, if not always, found in the epithelial cells of the gut-wall. It seems to me highly probable that the planont is furnished with an initial amount of energy which suffices to take it through the peritrophic membrane and into the gut-wall, there the initial energy gives out or the necessary stimulus to further advance ceases and the planont comes to rest. The organism after its wanderings are ended is called a meront. It is as a rule a completely rounded organism with one nucleus (Text-fig. A, 7).

What finer changes go on inside the planont during its wanderings are not of much practical importance, but it may be stated that the two nuclei which we noted in the organism in the spore seem to fuse together to form one nucleus either just before or just after leaving the spore case. At all events in place of two nuclei the planont has only one. Some workers profess to have discovered evidence that the planont in its wanderings before entering the gut-wall multiplies by dividing into two, which two again multiply by dividing into two and so on. I am much more inclined to believe that each planont instead of dividing into two fuses with another

* According to Stempell the planonts go right through the gut-wall into the "body cavity" and then turn back and attack the gut-wall—obviously a foolish proceeding and one which I cannot believe in. If they did this why are organs in the "body cavity" not attacked at the same time as the gut?

one, so that two planonts give rise to one. A discussion of this point, however, is of too purely scientific interest to occupy us here (Text-fig. A, 3–6).

When the planont—now to be known as a meront—settles down in the gut-wall it at once commences to divide. The nucleus first divides into two and then the body surrounding the two nuclei divides so that we get two organisms exactly like the original, only smaller (Text-fig. A, 7–11). These meronts go on dividing with great rapidity until the cell which the original planont entered becomes full of meronts (Text-fig. A, 12–14). It is not certainly known what causes the meronts to stop dividing—it may be lack of food or lack of space, some outside stimulus, or it may be some internal stimulus which determines when multiplication shall stop.* Whatever may be the cause, a stage in the development of *Nosema* comes when the meronts stop dividing. They do not spread to surrounding cells or tissues: they seem to remain in the cell in which the original planont came of rest.

The meronts now proceed to turn into spores, each meront forming one spore. A meront is round to oval in shape and has a single nucleus. When it is about to become converted into a spore it becomes a definite longish oval and a vacuole appears at one end (Text-fig. A, 15-16). The nucleus undergoes a series of divisions which need not be gone into here, and finally the thick spore-wall is secreted round the meront (Text-fig. A, 17). A second vacuole develops at the opposite end to the first one and the living substance, containing two main nuclei and several—up to 5—subsidiary ones, forms a girdle round the inside of the spore-case. We have then got back to what we started from —a ripe spore.

If the spore remained where it was formed it would not do much harm, nor would it accomplish much towards the multiplication of the parasites either inside or outside the caterpillar's body. The function of the spore in all the Sporozoa is to hand on the infection from one host to another. The thick spore-case is a protection against the drying up of the living contents while the spore is exposed to unfavourable conditions outside the body of the host in which it was produced. What then is the fate of the spore after it has been formed in the cell of the gut-wall of the caterpillar? If we were dealing with a very heavily diseased caterpillar there would be the possibility of its dying and the spores being set free by the decomposition of the body, but in the vast majority of cases this may not happen. How then are the spores to reach the outside and infect a fresh caterpillar? In order to understand this we must

* As spores and dividing meronts may be seen in the same cell (Plates III and VII, fig. 1), it would appear to be something more subtle than mere lack of space or food that brings on spore formation.

understand a little about the working of the gut-wall cells and the peritrophic membrane. These cells are continually budding off portions into the space between the gut-wall and the peritrophic membrane (Plates III and VII, figs. 3 and 1). These buds are supposed to be the bearers of the digestive juice produced by the gut-wall. When a cell is infected with *Nosema* it continues to bud off portions although very probably the production of the digestive juice is seriously interfered with. Thus the spores produced in a cell of the gut-wall may ultimately be shed into the space between the gut-wall and the peritrophic membrane in one of the digestive juice buds. Now the peritrophic membrane is a delicate tube attached to the gut-wall by its anterior end at the line of junction between the fore and mid gut (Text-figs. B and C). It hangs freely in the lower part of the gut so that the spores liberated into the space between the gut-wall and the peritrophic membrane can pass down this space to the rectum—or that part of the gut where the fæces are formed. There they become stuck to the *outside* of the fæcal mass as a rule and so pass out of the body. When a caterpillar is very heavily infected with pebrine and when the infection has been running for some time, the peritrophic membrane becomes very imperfect and full of holes so that the spores shed into the gut get mixed with the undigested leaf and so passed to the exterior mixed with the dejecta, but in the early stages of infection the peritrophic membrane is intact and the spores cannot and do not pass through it but reach the outside as has been described above.

So much then for a rough outline of the development of *Nosema bombycis* inside the gut of a silkworm. If the original infection ended with the transformation of the meronts into spores the parasite would never be the terrible plague that it can be. Even if hundreds of spores were eaten by a caterpillar only a relatively slight infection would result, for not all the spores dehisce in the gut and not all the planonts manage to reach the gut-wall. Thus only at most, say, a few hundred gut-wall cells would get infected, and as we have noted infection does not spread directly from one cell to another. This would be the limit to the infection unless the worm was constantly being reinfected. But this is not so: once a caterpillar is infected the infection spreads inside the caterpillar. How is this effected? It is obvious that what is known as auto-infection must occur, and careful investigation shows that this actually does occur.* The spores which are shed from the gut-wall cells into the space between the gut-wall and the peritrophic membrane may be ripe and ready to dehisce, in which case the necessary stimulus is present in the digestive juice, the spores shoot

* *See* Kudo (1916) on the subject of auto-infection.

out their polar filaments and the planonts issue from the spore-cases and attack the gut cells. This is the stage, I believe, when tissues and organs outside the gut get infected. The planonts which escape into the lumen of the gut at this stage do not have to penetrate the peritrophic membrane in order to reach the gut-wall. They are already in close contact with the cells of the gut. They thus readily penetrate the gut-wall, and they have sufficient initial energy—since it was not used up in traversing the peritrophic membrane—to enable them to make their way through the basal membrane and so into the cavities (hæmocœle) round the gut whence they can attack the different tissues and organs that lie in these spaces (Text-figs. B and C). Of course some planonts as before settle in the gut-wall cells. When these planonts come to rest, be it in the gut-wall, in the silk-gland, or in fat-body, they repeat the history we have already outlined: they multiply abundantly, forming nests of meronts which finally change into spores.

Now the reproductive organs lie in the hæmocœle, and therefore when planonts of *Nosema bombycis* enter this space these important organs may become infected. It is in this way that pebrine is an inherited disease. The sperms or "male seed" are not liable to be attacked: the testis, or organ which produces the sperms, may be infected, but the sperms themselves are either too tiny or are in some other way unsuitable, for I have never observed any disease in them or in the cells that produce them even when the surrounding tissues of the testes were heavily infected. The eggs, too, are not easily infected except in the young stages; the protective envelopes that surround the eggs develop fairly early and the planont cannot penetrate these, while the chance of a planont entering the micropyle of the egg is very remote. In the early stages of development, however—those stages which are passed in the caterpillar and early pupa—infection can and does take place.

Difficulty of observation here makes one cautious and the following statement must be regarded as only approximately correct and complete. On entering an egg the planont does not develop so rapidly as in other tissues. It seems either to lie dormant or almost at once forms spores: possibly the food substances present in the egg at this stage are not readily available and the parasite cannot obtain the required nourishment. The parasite is not, so far as my experience goes, found in any numbers in unlaid eggs: the most heavily infected pupæ can be searched with the greatest care without finding more than the very slightest infection in the eggs. After the eggs have been fertilized and laid, however, and once the eggs start developing, the parasite goes ahead for a time. It is probable, I think, that when the yolk of the egg is being made available for

the developing embryo, when it is being broken down or digested, a source of food supply is made available for the parasite and a stimulus is provided in the digestive enzyme which causes the spores already formed to dehisce. At all events dehiscing spores and developing planonts and meronts are to be found in infected eggs at this stage of development (Plate III, fig. 2). At the later stages of development in the eggs when the yolk is nearly all used up and when the embryo is nearly fully formed, meronts are no longer numerous, but spores have been formed in various parts of the embryo, especially in the gut, the hypodermis and what remains of the yolk (Plate VII, fig. 3). Infection may be so heavy that the hatching of the egg is delayed or totally prevented, but this would be only in extreme cases. As a rule a young caterpillar already pebrinised emerges from the egg. The infection is nearly always, at least partly, a gut one, so that the young caterpillar is capable of passing spores in fæces and so infecting other caterpillars, or of still further infecting itself by auto-infection (Plate VII, fig. 4).

There is another source of auto-infection which may possibly exist. Spores produced in various tissues or organs lying in the hæmocœle may possibly be set free into this space by the rupture of the cell in which they were formed. It is not altogether improbable that some of these spores may be acted upon by the blood which contains a digestive ferment, and so planonts may be set free in the hæmocœle. Pasteur believed that infection could be acquired through wounds—the contaminated claws on the feet of some caterpillars penetrating the skin of others over which they crawled and carrying pebrine spores into the " body cavity," where they dehisced. I must confess that this mode of infection appears somewhat unlikely, and experiments tried by me to test this theory have not succeeded, but it must also be noted that in some of my preparations of highly diseased caterpillars from diseased moths' eggs, spores and empty spore-cases and planonts have been found in the hæmocœle. The importance of this can hardly be ignored: it is an indication that spores probably do dehisce in the hæmocœle and this source of auto-infection cannot be neglected. As a source of original infection too, in the way Pasteur indicated, it cannot be quite ruled out of court although it would in any case be a rare one.

It is thus seen that in the vast majority of cases—if not in all—the original infection is acquired by eating food contaminated with pebrine spores. Infection is spread within the body of a caterpillar once it is infected by auto-infection. Infection is spread from one caterpillar to another by the spores passed to the outside in the fæces or liberated from a diseased caterpillar or moth on its death and disintegration. We have therefore now turn to the practical

application of the above life-history and see in actual practice how much infection is actually picked up by healthy worms in disease-infected houses, what the serious sources of infection are, what the duration of infection is, what means can be used to prevent infection and so on. But before turning to those questions there is one small point in connection with the above life-history that may be noted —the time in which the cycle can be completed, the life-cycle being from planont to spore.

In N. G. Mukerji's "Handbook of Sericulture" the portion dealing with diseases is somewhat imperfect and misleading—partly because in the day when it was written our knowledge of the subject was very rudimentary, but also because there is a considerable amount of faulty observation in it. The account of the development is very far from correct, but the most serious mistake is the amazing statement that "it takes about 20 days for the germs of pebrine to attain the corpuscular shape." The truth of the matter is that spores are formed in large numbers in an incredibly short space of time.* Some worms fed with spores on the first of September and killed on the fifth had their gut-wall cells full of spores as well as developing meronts, that is to say four days after infection spores had been formed in large numbers (Plate VII, fig. 2). In colder weather development is slower, but spores can always be found a week at longest after infection—of course they may not be numerous, that depends on the amount of the original infection. It is important to recognize clearly the rapidity of spore formation under ordinary conditions because much has been made of the necessity for delaying moth examination until the last possible moment. This, I believe, is not so extremely important as is thought, for owing to the rapidity of spore formation it is certain that if the worms were infected—and it is of course only the worms that can be infected—by the time the pupal stage has been gone through and the moth cuts out there will be a large number of spores present in the body. Indeed if one examines a large number of pupæ from infected worms one will see that spores are abundant but meronts are on the whole much less common. Thus when the moths emerge, if they are infected they ought to have sufficient spores present in their gut and other organs to show the infection easily. The pupal period is at shortest a week, so that if a caterpillar were infected on the day before spinning there would be ample time for the meronts to turn into spores before the moth emerged.

Amount of disease in India. It would seem to be the obvious thing in any inquiry into a disease of this kind to institute a census

* Stempell found spore formation after three days.

of disease. It is useless depending entirely for evidence on rearers and others connected with silkworm-rearing. It is to me amazing that so much has been written about the amount of disease present in the silkworm in India and so little has actually been done to find out the truth. I have endeavoured to take a census which was conducted as follows. Cocoons were collected from various nurseries and villages and forwarded to me. When the moths cut out, they were examined and the amount of disease was in this way directly discovered. The details of this investigation are given in Appendix I : some of the results as regards pebrine are given below. The results thus got were checked in two ways : (1) by questioning professional rearers from various parts of Bengal and (2) from the personal observations of myself and my assistants while touring at different times in the different silk districts of India. The answers given to my inquiries from the professional rearers are given in Appendix II. A general statement of the opinions expressed on pebrine will be given later, after the facts elicited by the first census are discussed.

The first sample of cocoons received gave most interesting and most typical findings. There were four lots—three of Chhotapolu and one of Nistari—and all that was known about them was that they had been reared in some villages near Berhampore in Bengal. The cocoons were all of poor quality and the Nistari were no better than the Chhotapolu. On examination the Chhotapolu were found to be practically 100 per cent. diseased—one lot was 99 per cent.—while the Nistari showed only 3 per cent. disease. Inquiries were then made as to the origin of the seed used in producing the cocoons, and it was found that the Nistari were from nursery seed while the Chhotapolu were from village seed—traced back as village seed for three generations (*See* Appendix I A, lot 1 ; and I B, lots 1—3).

And this is what has been found all through. When unexamined seed is used, heavy infections of *Nosema* are always got (Appendix I A, lots 17 and 19 ; and I B, lots 1—4). In this connection the hybrids in Appendix I C, lots 1—5, are most interesting. These were reared under exceptionally favourable conditions—they are not village seed—and yet the percentage of disease is very high. The nursery stock has been singularly free from disease and the cocoons reared from nursery seed are also very satisfactory (Appendix I A, lots 1, 5—7 ; and I B, lot 5).

In Appendices I G and I F, I publish extracts from the grainage registers of the Mysore Department of Sericulture—by the kind permission of the Superintendent—and of Berhampore Central Nursery— by the kind permission of the Deputy Director of Sericulture, Bengal. The former of these is very interesting as it con-

tains records of examinations of seed reared by the ryots themselves, reared by the ryots under the supervision of the department, and in the Government farms. The latter is a record of nursery stock alone. It will be found that *Nosema bombycis* is always present in a greater or lesser degree—usually not more than 6 or 8 per cent. as a rule, but sometimes, as in August 1919 in Berhampore, rising as high as 10 to 20 per cent. I am inclined to think that some of these higher percentages require an explanation: either there has been a great deal of infection in the rearing houses or the examinations made of the parent moths have not been very careful. I am of the opinion that the latter is the true explanation, as I have found by rearing very carefully examined seed in the Berhampore Central Nursery that little or no disease is picked up in the houses there (Appendix I A, lots 2—4, 10, 16 and 21). There is, however, obviously a percentage—somewhere ranging between 2 and 6 per. cent. —below which it seems to have been impossible so far to get infections of *Nosema* reduced in the nurseries.

I believe this percentage represents the amount of *Nosema* infection missed in the previous examination and not fresh infection picked up in the rearing houses. It can hardly be called " disease " for it has shown itself only in microscopic examination, and these moths are comparable in a way with the " amœbic carrier "—a person who may not have suffered from amœbic dysentery yet shows the organism in his stools. Such a percentage of infection could, of course, be eliminated or reduced to a completely negligible amount by very painstaking examinations. It is a question as to what will be profitable: a small percentage of *Nosema* infection at certain examination cost or a smaller percentage at an increased cost. In any case the amount of *Nosema* infection is not to be greatly dreaded, provided the seed is reared under reasonably good conditions and for one generation only.

In Pusa where experimental rearing has been going on for some time disease is practically non-existent. In hundreds of controls examined, the percentage of pebrine found is so small as to be absolutely negligible (Appendix I D).

Here then we have a considerable body of facts to go upon, and from these we can draw the following conclusions :—

1. In ordinary village stock pebrine is appallingly abundant.

2. In carefully examined, carefully reared stock, such as has been used for experimenting in Pusa and in Kalimpong, disease is practically entirely absent.

3. In nursery stock there seems to be relatively little disease—at best none at all, at worst about 8 per cent.

4. In nursery stock reared in villages there is relatively very little disease.

These are the results of our census and they are, I think, very encouraging. They prove several things, but first and foremost that in sericulture in India, as elsewhere, reasonably disease-free seedgives disease-free worms and moths. The method of seed examination in most of the nurseries in Bengal is not really good: the microscopic preparations are much too thick to enable one to see with any accuracy if spores are present in small numbers. While this may be so, it is doubtless much better now than it was a few years ago. But when all is said and done, the Bengal nurseries have not merited all the criticisms thrown at them. They are at present producing seed which is infinitely superior to ordinary village seed, and they have demonstrated that reasonably disease-free seed can be produced in the plains of India. In view of these facts I cannot altogether agree with Professor Lefroy when he states in his report that "For thirty years disease-free seed has been issued, but no disease-free race exists at present even. The scheme has manifestly failed." Something has been done, even if a condition of perfection has not been reached—this indeed was hardly to be expected. It is probable that improvement has only recently made itself evident, but improvement there is, and what is more important, it has been demonstrated that, even with imperfect means, progress is possible. All praise is due to Mr. A. C. Ghose, late Superintendent of Sericulture, Bengal, for what he has been able to accomplish in the Government nurseries. Much more remains to be done and it can be done better than it has been done, but pioneer work, such as he did, is always difficult, and credit for what has been accomplished must be given.

Infinitely more important, however, than any partial vindication of the Government nurseries in Bengal, is the demonstration that disease-free seed can be produced in India despite all drawbacks and that such seed when reared in villages picks up but little disease during one generation at least.

Such are the results of our examination of moths in the census. Let us now see what the general opinion of professional rearers is about disease. Naturally the statements of these men are rather vague and their experiences differ, but the following generalizations from the answers of the fifty odd men roughly represent, I believe, the experience of the rearers. Losses from disease have always occurred, about two *bunds* failing out of ten, but things seem to be improving on the whole, only one man—No. 6, Appendix II A—thinking that things, at all events cocoons, were worse now than previously, and in Appendix II C seven out of 20 rearers say pebrine is getting more common. Other diseases are, however, complained about.

The almost total failure of the November 1919 *bund* reared from Piasbari nursery seed is very remarkable (Appendix II A, rearers 2, 3, 7, 8 and 9), and it is interesting to note that this was said to be due to the issue of unexamined or badly examined seed owing to some mistake on the part of the nursery staff. The seed must have been extraordinarily bad to give such bad results, and it makes one wonder if the seed issued was really nursery seed at all. With the exception of this disastrous November *bund*, nursery seed seems to have given satisfaction, the maximum loss under ordinary circumstances from this class of seed being generally estimated at from $\frac{1}{16}$ to $\frac{1}{8}$ of the crop, while village seed occasionally gives a total loss. On the whole then it seems to be the opinion of professional rearers that pebrine is not increasing and that nursery examined seed gives good results. As the results correspond with those of the census it may be taken that they are reasonably accurate.

In the course of tours made in the silk districts very little disease of any sort has been found, and certainly no epidemic of pebrine has been come across. In the course of a tour in Mysore, the Superintendent of Sericulture there told me of a very interesting case of an outbreak of pebrine that they had had which was traced to a mistake having been made in packing some seed for distribution—the rejected seed having been sent out by mistake for the passed seed. In Kashmir one or two pebrinised worms were found in a lot reared from French examined seed. On the whole neither my assistants nor myself have found any appreciable amount of disease either in Government nurseries or in village rearers' houses.

Amount of loss from pebrine. It is practically impossible to form any idea of the actual losses that the rearers suffer from this disease. There are no records of any sort on the subject and one has to rely on the opinions of rearers. Occasionally one hears of a total loss such as that which seems to have occurred in Malda District in the November *bund* of 1919. On questioning the rearers they say that sometimes they lose the whole crop from disease but as a rule the loss is about " 2 annas ". This of course refers to actual deaths among the caterpillars. There is, however, a very serious loss in silk in cocoons spun by heavily pebrinised worms and this must be added to the total. On the whole I think it may be estimated with all caution that the village rearer never gets more than 75 per cent. of his silk crop. If he uses examined seed his losses from death among the worms will probably be slight but his cocoons may not be much richer in silk, owing to the underfeeding of the worms. There are so many factors to consider in writing of the silk yield—disease is only one of many. In Bengal the rearers seem to recognize that nursery seed gives better crops, and in Mysore very striking

results have been achieved by the Department of Sericulture. In two villages, Malurpatna and Hyndargoppa, where the silk industry was on the point of dying out completely owing to losses from pebrine, the department demonstrated that by rearing examined seed in one of the rearers' houses, which was thoroughly disinfected, a perfectly satisfactory yield could be got. In both places the industry has revived and the rearers, using examined seed, are getting full crops where formerly they could get none.

What are the common sources of infection? The results of our census of disease have led us to believe that the hereditary source of infection is very much more important than the contaminative but the latter is far from being negligible. A number of experiments were conducted to see what the percentage of infection was in caterpillars reared from diseased moths' progeny, what the percentage of infection was in caterpillars from disease-free stock reared in highly infected surroundings and also, in infected surroundings, what was the commonest source of infection.

HEREDITARY INFECTION. *Percentage of disease in diseased moths' progeny.* It will be readily understood that even if a moth is heavily diseased its eggs may not be very heavily infected, or indeed may not be infected at all. In this way the examination results in diseased moths' progeny vary very greatly. A laying may give as high as 100 per cent. disease or disease may be completely absent—although it must be admitted that these extreme cases are rare. Still no definite percentage of disease can be given as constant. If progeny from a diseased moth of the 1st generation diseased be taken during different rearing periods, the percentage of disease is found to range from about 10 per cent. to 32 per cent. (Exper. 1). If progeny from a diseased moth of the 2nd generation be taken during different periods, the percentage of disease is found to range from 16 per cent. to 69 per cent. (Exper. 2): in one case no disease at all was got, but as already remarked this is so exceptional that it is not included. If progeny from a diseased moth of the 3rd generation be taken during different periods, the percentage of disease is found to range from 49 per cent. to 96 per cent. (Exper. 3). Therefore it would seem that the more generations of disease behind them the higher percentage of infection up to a certain point.

In order to find whether there was any seasonal difference that might explain the differences in amount of disease between the different generations, lots 1 and 2 of Experiment 1 and lots 3 and 4 of Experiment 2 may be compared, as may lot 3 of Experiment 1 and lot 5 of Experiment 2. These lots from different generations of diseased moths' progeny had practically the same rearing periods, and yet it will be seen that in every case the first generation shows

less disease than the second generation. So that evidently the climate at rearing time is not of so much importance as the history of diseased generations behind them.

Very interesting results were got in Cambodia by breeding from diseased moths' progeny for several generations in succession (*Bull. Econ. de l'Indo-Chine*, No. 108, 1914). It was found that the disease gradually diminished, and that after six generations bred always from diseased moths only 1½ per cent. were diseased and these only slightly. It is certain that layings of diseased moths can be reared successfully if the rearing is good, but if they were subjected to the treatment meted out by the average rearer to his worms they would probably not be so successful. In Pusa I reared diseased moths' progeny for three generations without the worms dying out. Then I selected healthy layings from those that had three generations of disease behind them, and I found that they gave quite good results. Breeding from diseased stock for several generations does not seem to lead to any real weakening or degeneracy.

It is in this way, I believe, that epidemics run their course. Something induces rearers to over-production, or in some way to go in for bad rearing. Diseased moths are bred from and pebrine becomes rife, resulting gradually in almost the extinction of the worms in a district or country. Consequently production drops and the industry almost dies out. But probably the best rearers, who will be the most interested in the work, go on. There are always a few healthy worms somewhere among the diseased ones as we have seen. The diseased ones will gradually die out, and by careful rearing the few healthy ones will re-establish the race. Thus we can understand why despite pebrine the race of silkworms has persisted.

The above results show how high a percentage of disease is to be attributed to hereditary infection, and they also show what a serious source of infection to healthy worms even one or two layings of highly diseased worms might be.

WHAT PERCENTAGE OF DISEASE IS PICKED UP IN HIGHLY INFECTED SURROUNDINGS BY CATERPILLARS FROM DISEASE-FREE SEED? We have seen that the percentage of disease usually found in worms reared from diseased seed in healthy surroundings is very high; it is next necessary to see what amount of infection worms reared from disease-free seed pick up in highly infective surroundings. In the first instance an extreme series was taken. A small *kutcha* house was infected by sprinkling thickly over the floor, walls and roof, dust made by crushing the bodies of highly pebrinised moths. The resulting powder showed about 60 spores in the field of a microscope fitted with a Leitz $\frac{1}{6}$ objective and a No. 4 eye-piece. Worms

were put into the house on hatching and reared to maturity, the resulting moths then being examined for disease. Every day the floor, which was very dusty, was swept around with a brush so that the dust was raised in the air and might fall on the worms. The stand on which the worms were, was covered with dust. In short the conditions were extremely dirty and the dust was extraordinarily full of spores—infinitely worse surroundings than worms could possibly be subjected to in the poorest and worst rearers' house. The experiment was repeated five times, the house being freshly re-infected each time. The results are given in Experiment 4, Appendix III. As a rule the percentage of moths infected in the house was about 10 or 12 per cent.—that is to say, about the lowest that is got in hereditary infections. On one occasion the number of infected moths was high—40 per cent. —and it is to be noted that this was in the *bund* for the beginning of September. Even in exceptionally infective surroundings, then, it may be said that usually a heavy infection is not to be expected.

In order to arrive at an estimate of the amount of infection picked up by caterpillars from perfectly disease-free seed when subjected to normal infective surroundings, layings of very carefully examined moths were reared for me in Bengal by various rearers in their houses and by their own method. Cocoons of their own rearing from their own seed were also procured and examined. During the first of these experiments—Appendix IA, lots 8 and 9—the rearers had no worms of their own being reared while mine were being reared, and my worms, as will be seen, showed no pebrine. During the second experiment they were rearing their own seed at the same time and disease was got—in two cases very little and in two other cases 18 and 21 per cent. (Appendix IA, lots 12–15). When my seed showed about 20 per cent. disease their own showed from 60–90 per cent. When my seed showed little disease theirs showed 1–15 per cent. (Appendix IA, lots 17–20). I believe that infection was carried to my worms from their own diseased stock by the hands and clothes of the rearers—the method of carrying infection that Pasteur (1870) believed to be most important—it was not so much in the house itself. Thus under ordinary village conditions if we start with good seed very little disease will be acquired. It will be seen here again that hereditary infection is much more to be dreaded than contaminative, although that too is of some importance if diseased stock is reared alongside of healthy.

Other sources of infection. It seemed to me that the tray on which the worms were reared was probably a much more serious source of infection than the house itself, and experiments were made to test this idea. The series of experiments about to be noted are

to be taken as a pendant to the rearing of worms in the artificially infected houses: they are extreme cases unlikely to be realized under ordinary conditions. Small bamboo trays such as the rearer uses were smeared with a mixture similar to that used in infecting the houses, only the dust was moistened with water in order to make it stick. The experiment was repeated six times (Experiment 5, Appendix III). As a rule the amount of disease picked up was very great, rising as high as 100 per cent. on one occasion. The lowest percentage of disease was 15·8 and the average was over 50. There seems to be no doubt that the tray would prove a much more serious source of infection than the house itself.

The practice of manuring mulberry land with silkworm fæces might, it was thought, lead to the infection of the worms fed on the leaf from plants thus manured, if the fæces contained pebrine spores. The experiments—3 in number—which were undertaken to test this idea were rather unexpected in their results, and I must confess that they do not appear to me to be altogether trustworthy. Where the fault lies I am unable to determine, and I give the findings—Experiment 6, Appendix III—with this caution. The material used in infecting the plot was similar to what was used to infect the house and trays. It was certainly very heavily infective, but surely not sufficient to give a disease percentage of over 80 in two of the lots. The second lot gave a percentage of 11·4 disease and that is possible, although even in this case it is difficult to understand how the leaves growing on the bushes, some little distance above the ground, could become contaminated sufficiently to produce even this amount of infection. It is possible that in gathering the leaves they were laid on the ground and thus got soiled with the pebrinised material used for manure. I confess I expected to find only a very small amount of disease if any at all in these experiments, and I cannot believe that the percentages given express the actual amount of infectivity. That infection was picked up, however, seems certain, but I cannot understand the high degree. In view of the result of this experiment —doubtful though it is to some extent—it cannot be wise to continue manuring mulberry land with silkworm fæces.

Still another source of infection to be noted is diseased worms being reared along with healthy stock. A number of experiments have been done in the past which show that diseased caterpillars are capable of spreading the disease to healthy caterpillars when the two are reared together. Most of these experiments, however, have been done by feeding some caterpillars with spores and then mixing them with a healthy lot and the results have always given a high percentage of disease. Two experiments of this type are given

in Experiment 7, lots 1 and 2, the result being that 100 per cent. disease was acquired in one case and 40 per cent. in the other. It seemed to me, however, that perhaps the more normal source of infection would be diseased moths' progeny, and two experiments were tried to see what amount of infection would be picked up in this way (Experiment 7, lots 3 and 4). First and second generation diseased moths' progenies were mixed at hatching with disease-free worms. On examining the moths from these rearings in the first experiment—where 2nd generation diseased moths' progeny was used—the mixed lot gave 31·3 per cent. disease while the control of the diseased laying gave 16 per cent., showing that probably a fair amount of infection had been picked up. In the second experiment the mixed lot gave 11·3 per cent. while the control of the diseased laying gave 10 per cent., showing that relatively very little infection had been picked up by the healthy worms. These two experiments are obviously imperfect as in examination it was impossible to distinguish which were diseased moths' progeny and which were from disease-free layings. This could only have been done by the caterpillars spinning different colours or shapes of cocoon, and at the time none was available. However it seems clear that infection can be got from diseased moths' progeny but not to a very great extent in a very small rearing. Reference may be again made to the experiments in rearers' houses (Appendix IA, lots 12 and 15) where diseased moths' progeny seem to have acted as a source from which disease-free moths' progeny got infected.

There are other sources of infection such as air-borne spores and the clothes and hands of people coming from houses where highly diseased rearings are found. The former is certainly an unimportant source, and the second is well guarded against in this country as rearers object strongly to outsiders coming in and looking at their worms, especially when disease is about. Indeed so deep rooted is this feeling that it points to a past experience of highly contagious diseases extending back for a very considerable time.

How long does infection last? A large series of experiments on the length of life of pebrine spores is recorded by Hutchinson (1920). The results are rather irregular and the conditions under which the spores were kept not normal—the material being kept either continually moist or continually dry—so that they cannot altogether be accepted as showing what might happen under ordinary conditions. On the whole they seem to show that when spores are kept dry they retain their vitality longer than when they are kept moist, and that spores kept in a dessicator may remain infective for 5½ months. As, however, the atmosphere of the majority of the silk-rearing centres in India is not so totally free from moisture

as that inside a dessicator this figure need not cause excessive alarm. I attempted to reproduce actual conditions (1) by keeping the bodies of pebrinised moths exposed to the air but protected from dust, etc., and feeding this to caterpillars at intervals of 2 and 3 months; (2) by using infected *kutcha* houses and keeping worms in them until infection almost or completely stopped. Under normal conditions during the hot weather it seems as if material remained infective for at least two months but not for three (Experiments 8 and 9). In the rains, especially towards the latter part, the risk of persistence of infection seems to be greatest—infection being got in a house nearly eleven weeks after it was infected (Experiment 9, lot 2b). As the cold weather comes on the infection in the houses seems to be much less persistent (Experiment 9, lot 3b). On the whole it must be taken that under normal conditions we must expect material to remain infective for at least about three months—it may be shorter lived than this under some conditions but it is not safe to build on that. Unfortunately this period is just long enough as a rule to carry on infection from one *bund* to the next, so that the necessity of devising some method of protecting worms from infection, by disinfection or otherwise, is necessary in India. In France it is now recognized, as indeed Pasteur pointed out long ago, that the long interval between the rearing periods of the univoltine races makes disinfection for pebrine more or less unnecessary. Unfortunately the *bunds* of the multivoltine worms reared in India follow each other so closely that we cannot afford to neglect all precautions. If, however, a district that was heavily infected with pebrine could be given a six months' rest from rearing, that would probably be more effective than any disinfection. Unfortunately it would hardly be possible to do this. In Kashmir the rearers' houses are never disinfected but pebrine is of no importance there—univoltine worms are used.

Methods of protecting worms from infection. If unexamined seed is used nothing will avail—it would hardly be worth while to bother trying any disinfection or other preventative measures—but if good seed is used it is only right to give it every possible chance and try to eliminate every outside source of infection, however slight it may be, in every possible fashion. There are several different methods by which one may attempt to protect worms from infection. These fall into three main divisions:—

1. Direct protection from dust containing spores falling on the rearing trays and the worms thereon.

2. Destruction of the spores in the rearing house by germicides.

3. Increasing the natural resistance of the worms.

1. PAPER COVERS. In one of the highly infected *kutcha* houses some worms were kept under a cover made from newspaper (Experiment 10), and it was found that although the worms in the open trays gave 12·5 per cent. disease those under the cover were free from disease. The experiment was repeated with a similar result. Thus it would seem to be possible to protect worms by merely erecting a tent of old newspapers over them—by protecting them from any dust that might contain spores of pebrine.

2. DISINFECTANTS. The most common method of destroying infective germs is by using some germicide or by letting in the sun on them. The rearers in this country have great faith in cow-dung in this connection, and indeed there is a certain amount to be said for it. That it has any germicidal effect I do not believe (Experiment 13), but it serves to lay the dust—to fix the spores, if there be any, to the floor, the tray or whatever is smeared with it, and in this way may help to prevent the spores getting from the floor, etc., on to the food of the worms. The smoke from the domestic fire is also claimed to be a good germicide. Moveable objects such as trays in the rearing houses are usually exposed to the sun and it is reasonable to expect that this might have some effect. Experiments were designed to test this. Some pebrinised material was exposed to the sun for a whole day and then fed to caterpillars as leaf smears. The material exposed to the sun gave no disease, while the unsunned control gave 17·5 per cent. disease. Material kept in a closed wooden box, which was exposed to the sun, gave the same result as the material exposed directly to the sun (Experiment 11). Next two bamboo trays were smeared with infective material and one was exposed to the sun while the other was kept in-doors. This experiment was repeated five times (Experiment 12). On one occasion the sunned tray gave no disease while the unsunned one gave 15·8 per cent. In the other cases the sunned trays gave considerably less disease than the unsunned one. On the whole probably exposing the furniture of the rearing house to the sun is of some use and is to be recommended.

Solutions of copper sulphate of about 1 or 2 per cent. strength were long used as the standard disinfectants in sericulture, but within recent years this has been abandoned in favour of a solution of "formalin" in water. I understand that the Japanese use this, and the French sericulturists, so long wedded to copper sulphate, also have found formaldehyde more useful. I used three methods of testing these germicides: (1) infective material in the form of a powder was sprayed or otherwise treated with the germicides and the treated material was fed to worms; (2) trays and cages smeared with infective material were treated with the germicides and then cater-

pillars were reared in them ; (3) *kutcha* houses were heavily infected, and after being disinfected they were used for rearing worms in. The results of these experiments will be found in Experiments 13–15. On the whole, formalin, in the form of a 1 per cent. aqueous solution, was the most efficient, but it did not seem to kill all spores. In the infected houses it showed up very well, but when sprayed on material which was afterwards fed to worms it was evidently not so effective. Stronger solutions or heavier spraying of course produced an improvement. Copper sulphate was not of much use. On the whole, while the disinfection experiments were not very conclusive, they indicate that for routine disinfection 1 per cent. formalin in water—*i.e.*, one part of formalin (40 per cent. formaldehyde) in 100 parts of water—would probably be useful. If one were disinfecting after an outbreak of disease, however, the strongest solution that can be afforded should be used—say, anything from 2 to 5 parts in 100 parts of water. Lime-wash, although hardly a germicide, is to be recommended as it serves to lay the dust where it is applied, forming a skin over everything. Furniture that can be moved outside should first be well washed in 2 per cent. formalin and then after about an hour put out in the sun for at least a day, longer if possible. Particular attention should be paid to the trays—indeed after an epidemic these should be burned.

3. HEIGHTENING RESISTANCE TO DISEASE. The question of the heightening of the resistance of any animal to protozoal infection is a very thorny one, and one on which there is no fixed opinion. It may be useful, however, to pay some little attention to it here.

Let us look first at some general considerations. All living organisms exhibit, to some extent, resistance against parasitic attacks. This resistance is known as natural immunity. There is a second kind of immunity distinguished—acquired immunity. That is to say, after an attack of certain diseases, usually bacterial in origin, the animal attacked, if it survives, is immune to further attack. Some little progress has been made in the study of immunity in man and the higher vertebrates but practically nothing whatsoever is known about immunity in invertebrates, and it is never wise to argue that because certain things are so, say, in man, they will necessarily be the same, say, in insects. Then again even in man the resistance phenomena that have been studied nearly all relate to bacterial attacks, and the question of immunity to protozoal attacks is hardly at all understood, and our ignorance of the response called out in invertebrates by protozoal attack is still more profound. One must therefore treat the subject with the greatest caution, and the most that one can do is to suggest a few ideas on the subject. It is an untouched field and one that demands the attention of a physiologist.

While acquired immunity is of the greatest importance in the study of disease in the higher animals, it does not come into question at all in connection with silkworms. So far as we know, once a caterpillar is infected with pebrine, it continues infected until it dies—it does not recover and remain immune to further attacks. We have therefore only to deal with natural immunity.

In order to understand immunity one must know what injury the animal concerned has to protect itself against and how the protection is achieved. Parasites cause hurt to their hosts in several different ways which may be grouped in four main classes:—(1) Mechanical or the destruction of cells and tissues invaded by the parasite; (2) the "digestion" of the tissues of the host by the parasite; (3) the lessening of the normal food supply of the host by the parasite absorbing its food either from the digested food in the gut or from the food absorbed by the gut and distributed to the various tissues; (4) the production of waste products—toxins—which are poisonous to the host. The ways in which an animal, on the other hand, resists parasitic attacks are more subtle and difficult to determine but there seem to be certain very definite defences in the animal body. In addition to such obvious protection as, for example, the impervious skin and the action of the digestive juices afford, there are the active and very efficient wandering cells or phagocytes —minute freely moving cells capable of being hurried by the blood stream to any point attacked by parasites and there actually assailing the invaders, and if these are small enough taking them inside their bodies and digesting them or causing sloughs of infected tissue to be thrown off. Still more elusive but extremely potent defences are afforded by the germicidal power of blood and the ability of animals to produce substances—anti-toxins—which in some way neutralize the poisonous waste products of certain parasites—the toxins. Other means of defence there are, but enough has been given to show that animals are by no means powerless against attack.

We must here, however, stop to note an important feature of parasitism, remembering that we are dealing at present not with bacteria but with protozoa, namely, that the huge majority of parasites do relatively little injury to the animal in which they live and consequently the whole army of animal defences is not arrayed for their destruction. The efficient parasite is one which causes as little injury to its host as possible—it is one which in the long course of time has arrived at a *modus vivendi* with its host. When a parasite produces toxins that are fatal to the host or rapidly destroys large areas of tissues with fatal results, it is a clear sign that either the parasite and host are comparatively new to one another,

or that some new conditions have upset the normal balance which the two had established.

Now with these few general points in mind we may try to examine some of the features of the resistance of silkworms to *Nosema* attack. In the first place, what is the kind of injury caused to the caterpillar by the parasite? If one feeds caterpillars heavily on pebrine spores and after a few days examines them, they will show almost certainly no outward sign of disease, and yet when they are killed and their organs carefully examined it will be found that a caterpillar may have thousands of cells in the gut-wall packed with meronts and spores—half the gut may be infected—(Plates III and VII, figs. 3 and 2), while other organs of the body such as the nerve-chord, the excretory tubes (Malpighian tubes) and the silk-gland may also be seriously attacked. The parasites may be present in countless numbers, so that one at first cannot understand how the caterpillar could live for a day—much less be apparently quite well. The only and obvious inference from this observation is that at all events no toxin is being produced by the parasite; or to put it more cautiously, that the waste products of the parasite are not particularly deleterious to the caterpillar. It seems possible, as has been already mentioned, that the planont, at all events, produces some digestive ferment which helps it to make its way through the peritrophic membrane and through the gut-wall cells or other parts of the body, and some workers have supposed that the meronts also produce a ferment which helps them to break down the contents of the cell in which they live. The enzyme produced by the meront, however, must be very weak, as it does not enable it to break down the wall of the cell in which it is multiplying and so spread from one cell to another. On the whole it must be admitted that, whatever ferments are secreted or waste products excreted by *Nosema bombycis*, they do singularly little harm to the silkworm.

How then does the parasite cause the disease pebrine? It seems certain the damage caused is chiefly due to the first and third causes given above—mechanical injury and lessening of food supply. The gut-wall cells invaded are prevented from producing digestive juice and also from absorbing food by the great mass of parasites which fill them, and the parasites further absorb their food from the sheltering cell and so still further rob the host of its food (Plate III, fig. 3). Thus arises the typical symptom of pebrine, namely, the slow unequal growth of the worms (Plate IV): they are being starved, their food is not being properly digested nor can they make full use of what is digested. The excretory tubes may be attacked and the cells in them prevented from functioning so that

the waste products of the caterpillar's body are not efficiently removed, and the animal becomes sluggish and its growth is still further interfered with. The silk-gland (Plate II, fig. 1) may be so injured that the silk-secreting cells do not produce silk in any quantity and no cocoon or a very flimsy cocoon will result. In this way can be explained some of the most typical symptoms of the disease.

We may take it then that the caterpillar has not to cope with any very poisonous toxins produced by *Nosema*, but there remains the fact of the very serious mechanical injury and gradual starvation which the worm has to undergo. Does the caterpillar have any means of resisting this ? In the first instance the infection of the caterpillar seems to be relatively easily effected. That is to say, the spores of *Nosema bombycis*, if ingested by the worm, " hatch-out" in the gut and the planonts readily penetrate the gut-wall. So far from preventing infection the digestive juice of the caterpillar actually is the stimulus which causes the spores to dehisce. It has been suggested that some disturbance of the gut, such as increased acidity, may be necessary before spores can dehisce, but I cannot find any proof of this. A normal caterpillar has a very alkaline gut—tested by Clark and Lub's indicators it gives deep blue with thymol blue (pH=9·8)—Jameson and Atkins (1921). If a caterpillar from a batch with this highly alkaline, or normal, gut be fed with spores, the spores will dehisce and the caterpillar will become infected with pebrine. Further, exactly the same degree of alkalinity is got (pH=9·8) in a heavily pebrinised caterpillar, whose gut is full of spores and in which, be it remembered, auto-infection is going on, that is to say, spores are dehiscing and planonts entering the gut-wall. The normal gut is perfectly fitted for receiving infection, and there is obviously no question of the planont being in any way digested or otherwise injured by the digestive juices of the caterpillar.

It may then be asked if all pebrine spores swallowed by a worm will dehisce and set free the enclosed planonts to start multiplying in the gut-wall. If a series of caterpillars be fed an emulsion of spores and killed at various intervals of time afterwards from about 10 minutes after feeding to say 8 hours, it will be found that all the spores ingested do not dehisce—indeed in many cases the majority seem to pass through the gut unaffected in any way. This is of course due to the fact that the digestive juice of the caterpillar stimulates only " ripe " spores to dehisce. Ripeness is doubtless closely bound up with certain nuclear phenomena and as such cannot be gone into here, but it will be readily understood that in the development of the spore there comes a point when it is ready to

dehisce—when the polar filament is ready to be extruded and the planont is ready to emerge and infect the gut-cells. Before this point is reached the digestive juice of the caterpillar has no effect on it and the spore in this unripe state would pass through the gut unaltered. An attempt was made by Hutchinson (1920) to count the unmber of spores that a caterpillar had to ingest before it becomes infected with pebrine, but his method was rather rough and the number obtained over 5,000 is much too high in all probability, unless he was dealing with a batch of spores that had a very great many unripe ones present. I attempted the same thing by feeding definite quantities of various emulsions and actually counting in a hæmocytometer the number of spores that would be thus ingested, and I found that 400 spores produced a heavy infection. Allowing for unripe spores and planonts which failed to reach the gut-wall it obviously does not need a large number of spores to secure infection—another demonstration of the fact that a caterpillar seems fairly easy to infect.

Granted then that there is no great difficulty in infecting a caterpillar—that there is no great resistance to the initial attack of the parasite—how does the caterpillar act towards the parasites once they are established? We have seen that the parasites multiply with great rapidity—the gut can be infected from one end to the other in four or five days (Plate VII, fig. 2)—and that the worm is tolerant of an enormous infection. Does this mean that here too the caterpillar has no very adequate defence against *Nosema* attack? The mere fact that the parasite can multiply so rapidly and be present in such enormous numbers would seem to show that the more passive defences are unable to hold this particular parasite in check to any extent. There are, however, two indications that the caterpillar does react to a heavy infection to some extent. We have already noted the budding off of part of the gut-wall cells as digestive juice buds. This, while leading to auto-infection, also helps to throw off a certain number of parasites, for the buds will contain many meronts and many unripe spores which are not capable of producing auto-infection, and which will thus be passed to the outside. In certain cases of very heavy infection I have observed what appears to be an intensification of this process. The epithelial cells in large areas of the gut-wall had budded off portions to such an extent that they were reduced to the merest fragments fringing the basal membrane. The gut thus presented a most curious appearance, looking as if it had attempted to throw off the parasites by budding off the epithelial cells that contained them. A much commoner response to *Nosema* attack is seen in the blackish spots which have given the name to the disease pebrine (Plate I, figs, 2, 4, 5). Their origin seems to be as follows:—Foci

of infection in the hypodermis or inner layer of the skin (Text-fig. B) become gradually surrounded and infiltrated by a deposit of chitin—the hard, non-living substance that forms the outer skin and all hard parts of insects (Plate II, figs. 3 and 4). It is a sort of cyst-wall being formed round the nest of parasites—the parasites are enclosed in an impervious, dead cyst and so prevented from doing further injury to the host. This is a common response of insects to parasitic attack. These black spots—or dark brown rather because chitin in mass is that colour—are in India found chiefly in caterpillars from diseased layings, as indeed the hypodermis does not as a rule get infected very heavily with the parasite unless it acquires its infection in the egg. At times, too, minute black specks are found on the outside wall of the gut of caterpillars that have been very heavily infected with pebrine (Plate II, fig. 2). On examination these are seen to be of the same nature as the spots in the skin—chitinised cysts containing pebrine spores.

Such then is a short review of the facts that we can observe regarding the resistance responses of the silkworm to attacks by *Nosema*, and it must be admitted that the natural immunity of the caterpillar does not seem very effective in preventing or arresting the onset of this particular parasite. But if we consider a moment this is hardly to be wondered at. Reference has already been made to the probable, almost certain, antiquity of the association of the caterpillar with *Nosema*. In dealing with the history of the causal organism of pebrine it was argued that *Nosema bombycis* was an old established parasite of the silkworm and probably was present in the silkworm long before its domestication. If this be so it explains certain points that we have been dealing with above. We have seen that it is characteristic of an efficient parasite that it should not under ordinary circumstances greatly injure its host—especially that it should not produce toxins—and that in turn the host should be tolerant of the parasite. It might also be added that in a case of parasitism such as a wild *Nosema* infection it would be essential for the infection to be easily acquired, for under normal conditions the risk of infection would not be great and if infection were not readily accomplished the parasite would tend to die out. All these conditions are really found in pebrine. The balance established between the host and the parasite in the distant past is in the main lines still preserved. *Nosema* easily infects the caterpillar and in mild infections does relatively little harm, while in turn the caterpillar is very tolerant of the parasite and does not, so to speak, resist it in force. The changed conditions, however, from wild life to domestication have enabled the parasite to infect the host much more heavily than it could in nature, and the environment under domestication is not so favourable to the caterpillar, so that probably

the infection may spread more rapidly in the caterpillar's body. In this way the parasite seems to have rather the upper hand and the caterpillar has not been able, so far as one can see, to cope with the parasite very efficiently when present *in large numbers*—so long as infection is slight the original balance seems to be maintained.

It is therefore very essential that we should determine what factors, if any, are likely to help the caterpillar to resist this mass attack. Now, it is a well known fact that immunity in men—who are animals singularly susceptible to disease—and other mammals varies according to individuals, according to the health of the individual at the time of attack and also to countless and often very minute variations in the environment—such as in temperature, moisture, amount and kind of food, etc., etc. In absence of evidence to the contrary we may presume that silkworms are similarly constituted. We may inquire first if it is possible to discover more resistant races of caterpillars and by selecting from them or using them in hybridizing experiments to evolve a highly resistant race, and second what are the conditions under which silkworms are most healthy and what can be done to secure these conditions and so raise their resistance by improving their surroundings.

Personally I think the search for a resistant race, or at all events a race more resistant than the local Indian ones, will not be crowned with success. These races have been bred for many generations under very trying circumstances, and it is reasonable to suppose that the fittest and most resistant worms have survived and form the present stock. It is significant that all attempts at introducing fresh stock have failed so far as one can find out from the records—the foreign races have not been able to stand the climate and the treatment meted out to them. That even European varieties can be reared with care in the plains of India is true, but that does not invalidate the above statement, for they could not survive one season under the ordinary rearing conditions in the villages. That introduced races fall especially a prey to pebrine I do not say—it is indeed usually flacherie or some similar rot disease that carries them off. In point of fact while I believe that worms vary very greatly in their resistance to rot diseases, I think the evidence shows that they are all more or less alike in their resistance to parasitic diseases. It is claimed by Mukerji (1899) that the Chhotapolu is more susceptible to pebrine than the Nistari and both more so than the Barapolu, but Mukerji's evidence is far from convincing. He also states that dark coloured or black caterpillars are more vigorous than the ordinary races, citing Cleghorn's black Barapolu. An exactly similar worm seems to have been selected by

Hutton about 1860. It was tested by Dr. Bonavia in Oude and did not seem to give outstanding results (Watt, 1893). If both Barapolu and Nistari tend to give numbers of dark worms under ordinary conditions (Mukerji, 1899) and if these are more resistant, how is it that the exacting natural selection always at work in the rearers' houses has not produced a dark race, the lighter worms being eliminated? I would not for a moment shut out the possibility of selecting more disease-resistant races from those already in India and an inquiry might be made into this subject as a sort of side line, but it seems to me that the results got will deal with so many varying factors and such slight differences that it will be well nigh impossible to form a correct judgment upon them. As I have said, a pretty exacting selection has been going on for generations in India, and it will mean several years of careful experimenting before we could claim that we had improved upon the results already achieved. We must also take into consideration the fact that in dealing with pebrine we are concerned, as shown above, with a parasite and a host which in the past achieved a balance—the attack of the parasite equalized by the resistance of the host—and that balance has probably been disturbed not by any alteration or variation in the inherited resistance of the worm or attacking power of the parasite, but by an alteration in the environment due to domestication. Therefore the most profitable method of assisting the caterpillar to resist attack will be to remove these disturbing external conditions as far as possible. Mukerji very pessimistically remarks, "if this (the discovery of pebrine in wild silkworms) be a fact, it is useless attempting to resist the plague by improving the vigour of silkworms." Well, *Nosema bombycis*—or at all events an organism indistinguishable from this—has been discovered in *Atherœa* sp. (Appendix I E). It has also been found in caterpillars of the genus *Gastropacha* and *Zygœna*, and I have infected a wild caterpillar (genus?) with pebrine. But I would not say "it is useless attempting to resist the plague by improving the vigour of silkworms." I would however tend to lay very much more stress on improving external conditions than on increasing the inherited resistance of the worms.

If the attempt to improve natural immunity through selection is not very promising, I do not think much progress is to be hoped for from hybridizing, for there of course one must have certain definite characters to work with—blind crossing, mere juggling with the germ plasm, will never lead anywhere. Here the opinion of a trained geneticist is essential. In Bengal a very considerable amount of hybridizing has been attempted, on the supposition that the Nistari when crossed with a better silk-yielding moth, such as a European or Japanese, would supply vigour or resistance to the cross—it is taken for granted that the Nistari has this character in

some particular degree—the object of the crossing having been of course to produce a vigorous worm that produced better silk than the Nistari. It is outside the scope of this investigation to go into the question of hybridizing, but it seems to have been undertaken in the most light-hearted fashion without any preliminary inquiry into the various characters of the worms and the ways in which they were inherited—on the whole a rather haphazard business. Now as we shall see later, the Nistari does seem to be resistant to rot diseases and is in general a very sturdy worm, but no experiments seem to have been conducted to see how far this was true and to what extent this was a heritable factor. Similarly with the richness of silk, which it was desired to get from the foreign worm, no work was done to see in how far this depended on climate, food, etc., rather than on some factor in the germ cell of the foreign worm. So far as I can see the results have not been more satisfactory than one would have expected from the methods employed. The best hybrids are a little better than the best Nistari at certain periods of the year, but they seem to be gradually deteriorating. In connection with this the observations of Grangeon (1917) are of the utmost importance. He decided that it was impossible to combine hardiness with richness in silk. He found that on first crossing Nistari with European or Madagascar worms, worms which gave a very good yield of silk were got, but in the succeeding generations the richness becomes less marked and the worms finally divided up into two classes—a small resistant worm and a large worm with less resistance which died out. From this it would seem as if the attempt to increase the resistance of worms by hybridizing was not likely to be a success. It may further be noted that almost certainly the quantity and perhaps also the quality of silk in a cocoon has more to do with climate than anything else (Coutagne, 1902). Much work on tropical sericulture has been done in Madagascar and the opinion of Fauchere (1913) is all in favour of this view. His contention is that silkworms are to be regarded as of tropical or sub-tropical origin and that the multivoltine condition is the normal one, the univoltine condition being artificially induced by rearing in climates of very variable temperature, but that very hot and moist climates do not allow of production of first quality silk.

Before undertaking any selection or hybridizing work, whether to improve resistance or to increase the yield of silk, the work that has already been done on sericulture in the tropics should be very carefully considered and then preliminary work should be undertaken to determine what is due to environment such as climate, food, etc., and what to actually inherited characters. When the ground has been thus somewhat cleared it will be seen whether any-

thing can be achieved in this direction. In any case such work can only be profitably undertaken by one trained in genetics—the subject is much too specialized for anyone else to make a success of it.

What seems to me a much more profitable line of work is the attempt to determine what external conditions help or hinder the caterpillar in its resistance to disease. Here we are dealing with external factors which are always varying a little from year to year, from place to place. In consequence of this the details would have to be worked out for different districts, and experiments would have to be continued for long periods. It is work which should be undertaken by the sericultural departments of the different provinces interested in sericulture—only the broadest outline can be attempted here.

It may be taken for granted that anything that tends to improve the general condition of silkworms will make them better able to resist the attack of *Nosema bombycis*. Improvements in environment should really aim at making the surroundings healthy, as like open air conditions as possible, for we have repeatedly emphasised the fact that under wild conditions it is practically certain that the host suffered little or no inconvenience from the parasite.

What then are the conditions in nature that we can hope to imitate under domestication? There are three main features of the wild life of caterpillars that we can try to copy.

1. *Comparative freedom from contaminative infection.* Things in nature are comparatively clean. Infective material is at once exposed to the action of the sun, bacteria, various scavenging animals, etc., and thus soon is rendered innocuous or removed. Besides which, as caterpillars are not crowded as a rule very closely on their food plants, and as the plants themselves are scattered, infective material is distributed very sparingly over a large area and the risk of mass infection is reduced almost to a quite negligible factor.

A great deal can be done by rearers to secure in their houses at least an approximation to the above conditions. The danger of infective material can be considerably lessened by the careful removal of all fæcal matter and litter in general and the storage of the some in a pit, where it should be mixed with other manures for the fields, and if the pit contents are dry they should be sprinkled with water to prevent the formation of dust which could be blown about. The litter should not be fed to cattle nor stored for burning, as it must be kept dry for these purposes and the dust danger is to be feared. Nor should manure containing silkworm fæces be used to

manure mulberry land. All dead caterpillars or moths should at once be burned. All moveable furniture in a rearing house should be frequently exposed to the sun and the house and furniture should be sprayed with some germicide—preferably 1 per cent. formalin and lime-wash every *bund*. The use of cow-dung for smearing trays, floors, walls, etc., is not to be discouraged. The place of rearing and its surroundings should be kept as clean and as dust-free as possible. The worms should be given as much space as possible—overcrowding is very harmful (for the results of overcrowding as seen in weight of cocoons see Appendix V, Graph 5.)

The question of overcrowding has always been a difficult one in India, and it is not being too exaggerated to say that as long as overcrowding goes on disease will be more dangerous and crops, whether diseased or not, of poor quality. Unfortunately it seems to be impossible to get rearers not to overcrowd. According to Mukerji, "they understand very well that it is healthier to have only 8 or 10 *dàlàs* on a stand instead of 16 or 17, and only $1\frac{1}{2}$ to 2 *kàhàns* (1 *kàhàn*=1280) of full grown silkworms, instead of 2 to 3 *kàhàns*, in each *dàlà*; but they will never abandon their practices."

It may be noted here that while the risk of contaminative infection is slight, the possibility of hereditary infection is considerable in the wild state. And here the domesticated worm may be said to be actually in a better position than the wild one, for it is possible to practically eliminate hereditary infection by a careful microscopic examination of the parent moths, so that domesticated worms may thus start life actually in a better position than wild ones.

2. *In nature there would always be a rapid and efficient circulation of air and abundant light.* The question of ventilation is probably the most difficult thing that the rearer has to face. It is rarely efficient in this country. The large houses in the Government nurseries in Bengal are very stuffy in the hot weather and rains, and the rearers' houses are distinctly worse. The matter is admittedly difficult, for while air is to be admitted as freely as possible the silk-fly and dust are to be kept out. In the Government nurseries there are special fly-proof wire-gauze doors and gauze-covered ventilators, but these last are altogether too small and the houses are very dark. The *kutcha* thatched house is said to be expensive in the long run on account of the frequent repairs to roof, etc., but on the whole they are cool, and instead of launching out into new types of houses, especially *pukka* houses, as has been suggested, it would be advisable to try to improve the existing type of house. This could be effected to some extent at least by not running the wall right up

to the roof but leaving a space of a foot-and-a-half or two feet between the top of the wall and the slope of the roof. This could be covered with gauze, the roof of course being supported by pillars or posts running up above the top of the wall. As these openings would be protected by the eaves they would not be exposed to the force of the dust-bearing wind, but if it was thought necessary light shutters could be provided to close when necessary. In French Indo-China it was found that rearing houses constructed on the European plan —*pukka* houses in fact—failed signally, while houses on the native plan succeeded admirably (Vieil, 1906). It would be folly after the attempts made in Indo-China—and there were several—to introduce that type of house into India. The only thing to be advanced in their favour is that they are more easily disinfected and kept clean than *kutcha* houses, but as these are not the hot-beds of infection they were thought to be, that argument is not of much worth.

While it is undoubtedly possible to improve the circulation of air in the rearing houses in the Government nurseries, it is a very different question in the rearers' houses. When new houses are being built under the supervision of a sericultural department the matter is simple, and more air and light could easily be obtained as has been above indicated ; but in the houses already erected, especially when they are also used as dwelling houses, probably very little can be done. However, the question of improved ventilation is one which should always be in the forefront of the programme of any sericultural department and it is one which can best be decided locally.

The darkness of the houses is also to be deprecated. It is said that on account of the fly the houses must be kept dark, but it is quite likely that the eye has little to do with the finding of the caterpillar by the fly and the laying on it of the egg. Before the fly could possibly see the worms—while the fly is outside and the worm inside a house—the fly can find out the worm. It must do this by some sense other than sight, so that even in a dark house the worms might not escape. Light in moderation is certainly not harmful to caterpillars, and a beginning should be made in the Government buildings, at least, to dispel the tomb-like blackness of the rearing houses.

In connection with the subject of ventilation it may be noted that it seems to be moisture in the air that is most to be dreaded. As will be seen from Experiments 16 and 17, when caterpillars were reared in a very moist atmosphere they were much more liable to disease than when the air was dry, although in the moist cages the caterpillars grew much faster and larger than in the dry ones and

made better cocoons on the whole. It is possible that the reason for the heavier infection is due to some lessening of the resistance of the caterpillars, owing to some metabolic disturbance caused by the inability of the worm to get rid of water vapour quickly enough, and it is significant that worms reared in warm, damp surroundings have a less alkaline gut-content than those reared under dry and hot, or moist and cool conditions—giving a grey-blue colour with thymol blue (pH=8·8)—*see* also under flacherie. It is also possible that the leaf being kept moist longer the caterpillars actually eat more and so ingest more spores, while the moisture would tend, at all events in the case of infected cages, to make the spores stick to the leaf while in the dry atmosphere they might tend to fall off the leaf. Whatever the exact cause of the greater percentage of disease, it is clear that a moist atmosphere along with a high temperature is very unfavourable, and a rapid renewal of the air in a rearing house is all the more necessary in hot, damp weather. It is interesting to note that the rains and especially August and September are very bad months for pebrine. This is the experience of the village rearers (Appendix II), and it is well borne out in many of the experiments (Experiments 4, lots 4 and 5; experiment 5, lots 3 to 5; experiment 7, lot 1, etc.). Now in these months the moisture content of the air is very high, and as breaks in the rains are frequent the temperature at times is very high, so that we have the same conditions as those reproduced in our experiments with the same result. August and September are the worst rearing months for disease and should be avoided if it is at all possible.

3. *In nature the worms would not be underfed, the food supply would be always fresh, and the mulberry eaten would be of the tree type.* The food supply of the silkworm is of the greatest importance. It is really just a perambulating stomach and responds wonderfully to good feeding. If worms are underfed or improperly fed they succumb much more readily to disease, and even when no disease is present the results may be almost as bad. Experiments 18 to 20 give some idea of the effects of proper and improper feeding. The first experiment with normal worms reared in infected cages is not so striking as the second one, although in one lot the underfed worms contracted pebrine while the well-fed ones did not. The next, however, is very impressive. Diseased moths' progeny of the first generation were divided into 3 lots and fed four times a day and six times a day on bush mulberry, and six times a day on tree mulberry. Those well-fed on bush mulberry were infinitely superior to those under-fed, and those fed on tree mulberry were vastly superior to those fed on bush. Plates IV—VI showing the appearance of the worms just before spinning, and the weights of cocoons, although these are of course at best poor, and the

extraordinary difference in caterpillar mortality given in Experiment 19, lots 1*a*-1*c*, are conclusive proofs of the value of the right food and enough of it. The second lots in Experiment 19 show the same results for rather different materials—here again generous feeding showing great improvement in the worms and the cocoons. The last experiment of the series was done on healthy caterpillars, and here the effect of underfeeding is seen in the slower and somewhat irregular growth and the poor cocoons produced. The effect of dirty leaf is shown in the same way but especially in the poor quality of the cocoon. The interesting point in this experiment being that the improperly fed ones gave quite the appearance of slightly pebrinised worms—slower, rather irregular and poor cocoons—if to this had been added overcrowding, the result would have been a product similar to what the average rearer gets and which is frequently put down to the fault of disease.

Thus it will be seen that sufficient food especially of a certain quality is of immense benefit to the silkworm. If the ryots would only feed their worms more liberally they would get better cocoons and, if there was no disease among them, a better yield of cocoons. If they could only be induced to use a little tree mulberry the results would be still more satisfactory. In this connection it is important to note that the good effect of the tree mulberry was not very noticeable until after the 4th moult, so that if caterpillars could be " finished off " on the leaf of tree mulberry the benefit would be great. It is further to be noted that the so called " trees " used in my experiments were only a stem of a bush plant which was allowed to grow up for about a year, while the other stems were kept cut, so that there is no real difficulty in getting tree leaf. With regard to more frequent feeding it may be mentioned that in Madagascar the young caterpillars are fed 8–10 times a day, older caterpillars 6 times a day and in the last stage they are never left without leaf. In Bengal about 4 or 5 times a day is the most they are ever fed and they are frequently seen with nothing but withered leaf or bare twigs on the trays. In Mysore the worms are fed at least 7 times in the 24 hours, including 3 feeds at nights, and they certainly show the benefit of the extra care.

Hill amelioration. In most sericultural countries, especially where univoltine worms are reared, it is considered beneficial to use seed which has been bred in the more elevated parts of the country. It has been suggested, therefore, that in order to supply the plains of India with seed, stations should be established in the hills where this " hill ameliorated " seed would be produced. The question is a very difficult one, and one which cannot, I think, be determined off-hand. It is certain that the hot and moist valleys of the Ganges

and other Indian rivers are not ideal silk-rearing districts, and the climate there would seem to make the worms more liable to suffer from disease. It is also certain that worms reared in the hills at an altitude of about 4,500 feet or somewhat higher do very well. They are not free from disease but they usually spin good cocoons and seem generally healthy. The point to be decided is, does this improvement got by rearing in the hills persist after the worms are taken to the plains—is it something that is inherited? Personally I very much doubt it, and it is a question that cannot rapidly be decided—several years of careful experimenting would be required to determine whether or no there is anything in hill amelioration.

Hutchinson (1920) gives a set of experiments which he says opens up " a hopeful line of enquiry " with regard to " increased resistance to infection caused by hill rearing," but I confess I cannot see on what grounds he bases his optimism. There are five experiments in the set. In one there is practically no difference between the hill reared and the Pusa reared worms—94 per cent. diseased as opposed to 100 per cent. In another the hill worms were fed on material 7 weeks and 5 days old while the plains worms were fed on material 4 weeks and 3 days old—such a difference in age of infective material as would easily explain one lot showing no disease while the other lot showed 30 per cent. disease. A third lot gave 40 per cent. disease in hill reared worms and 74 per cent. in Pusa reared stock, but if one examines the whole series of experiments given one finds that in the set on " the effect of artificial infection a day before spinning ", from which the 74 per cent. case seems to be taken, there is a case which gave only a 38·5 per cent. infection in Pusa, actually less than the 40 per cent. got in Shillong. A fourth experiment in infected cages gave 35 per cent. infection in the hill reared lot while the Pusa reared ones gave a 44 per cent. infection—a comparatively slight difference which is well within the limits that are to be expected from the chances for and against worms picking up infection in an infected cage. In a fifth experiment the hill reared worms gave 59 per cent. infection on being fed four meals of infective material after 4th moult while plains reared ones gave 100 per cent., but if all Hutchinson's experiments are reviewed it will be found that under the heading " the effect of artificial infection after the fourth moult," from which these experiments are taken, some lots of plains worms gave no infection, others 10 per cent. and 37·5 per cent.—again less than the hill reared worms. For my part I can see no support in these experiments for the belief that hill rearing increases resistance to disease.

I find, indeed, in the literature expressions of opinion which are opposed to hill reared seed being used in the plains. Pasteur

(1870) records that the seed got from the district of the Basses-Alpes did much worse in the plains of Gard and Ardèche than in the mountainous parts of these departments where the climate was similar to that in the Basses-Alpes. Geoghegan (1880) also quotes from an account by Lieutenant Powlett of Jaffir Ali's silk-rearing establishment at Gurdaspur in the Punjab—sometime about 1864—the opinion of Jaffir Ali that "acclimatized eggs were far better than hill raised, as the latter produced in the plains sickly worms." The Deputy Director of Sericulture of Bengal in a conversation with me has expressed the view that hill reared seed—and he has had experience of it for several years—does not do so well in the plains in the hot weather as plains seed.

It is extremely difficult to devise really good experiments to test the benefit of hill amelioration: there are so many varying factors. I have endeavoured to test this point, but I am fully aware of the faultiness of my experiments. Seed produced in Kalimpong has been sent down to Berhampore every *bund* and reared alongside of seed produced in the plains. Seed produced in Kalimpong has been sent to the plains (Pusa) and reared in an infected cage alongside of Pusa seed in a similarly infected cage, while a control of the Kalimpong seed was reared in a similarly infected cage in Kalimpong. As will be seen from Experiment 21 the results of the experiments in the infected cages were not in favour of hill reared seed. And the diagrams given in Appendix V shew that at least as far as weight of silk is concerned the hill reared stock was not so good as the plains acclimatized stock in some cases. In Appendix I will be found the results as far as disease is concerned of hill seed reared in the plains and it will be seen that in rearers' houses it may pick up quite as much disease as plains stock.

On the whole I cannot say that a case has been made out for hill amelioration as a whole. To reach any definite conclusion, however, would need many years' experimenting—indeed all experiments on silkworms should run for at least five years to be of real value, as the worms are so susceptible to climate and food that a few experiments cannot show absolutely definite results. There is, nevertheless, room, I believe, in each province for a small, cheaply-run hill station where seed for the cold weather *bunds* could be reared during the hot weather, where acclimatization experiments could be carried on, and where further experiments in the resistance of worms especially to rot diseases might be tried. To launch out into a scheme involving a big hill station would at present be folly.

Do conditions in India make the disease specially dangerous or difficult to check? We have reviewed above the sources of infection and the method of keeping pebrine in check, and we

may now enquire if there are any special conditions that make pebrine more difficult to control or in general more dangerous in this than in other countries.

There are three factors to be considered : (1) the parasite, (2) the worm or host and (3) the environment. The question of the parasite may be dismissed in a few words. The organism is same in Europe, India and Japan, and it runs the same course in the caterpillar. The higher temperatures got in tropical and subtropical countries will however tend to make its development more rapid than in cooler countries. According to Vieil (1906), this is a decided advantage and in the past prevented the complete sweeping out of the silkworms by pebrine in French Indo-China. According to this worker, the rapid development of the parasite leads to the death of the more highly diseased worms before spinning, or if they do spin prevents pupation, so that the diseased worms are eliminated before the time for laying eggs. In short the intensity of the disease lessens the risk of hereditary infection. But as the life-cycle of the multivoltine worm is much more rapid than that of the univoltine worm in temperate climates, I should have thought that this would tend to more than outweigh the more rapid development of the parasite. According to others, the rapid development of the worm in the tropical countries prevents the life-history of the parasite being completed and so the disease is less serious than in temperate countries.* I think it is certain that the disease is less devastating in the tropics than it was in Europe, and I believe that this is to be explained by the rapidity with which the worm goes through its life-cycle. There is less time for auto-infection to spread the disease all through the body of the host.

The silkworm of the tropics is nearly always multivoltine. Indeed it has been shown in Madagascar by Fauchere (1913) that the univoltine worm of the temperate zones becomes gradually multivoltine in the tropics, and from this it is argued that the univoltine state is an artificial one produced by transferring the silkworm from the equable tropical climate to a climate of very variable temperatures (European). The fact that in Bengal there is a univoltine worm does not altogether disprove this contention, as the climate of Bengal is not strictly tropical but has a considerable range of variability. The multivoltine character of the Indian or tropical worm must certainly have some effect on the question of disease. The Nistari worm may be got to produce as many as 9 generations in a year

* In 1913 it was said that pebrine in French Indo-China made much less havoc than it used to do in France and was decreasing. In Cambodia in 1914 it was said to be less serious and showed no sign of wiping out the worms. In Madagascar in 1917 pebrine was said to be serious partly on account of the carelessness of the native rearer, partly on account of the rapidity with which one generation succeeds another.

(Banks, 1911), but as a rule in India about six *bunds* are got —March, April, May-June, July, August-September, October-December. The rate of development of the worms varies very greatly according to the time of year, that is to say, according to the temperature. The eggs take from 7–12 days to hatch, the caterpillar stage lasts 3 to 4 weeks, and the pupal stage 10–16 days. The moths couple very shortly after cutting out. The whole cycle takes from 5 to 8 weeks. In the univoltine worm the life-cycle lasts a year—the eggs stage about 10 months, the caterpillar stage about 5 weeks and the pupal stage about 2 weeks.

Now in the first place the constant succession of broods throughout the year in India undoubtedly makes it more difficult for the sericulturalist to keep disease at bay. The rearing houses are never long enough vacant to let any pebrine spores that may be present in it die out—we saw that at least 3 months were necessary for this—and consequently disinfection must be resorted to. The short space of time between the laying of the egg and its hatching is also at least inconvenient, for it leaves a very short time for moth examination—days only where in Europe they have months. A great deal is made of this in India, but personally I cannot see that it is so very serious a matter, as I do not believe that the supposed increase of spores in the moth is of much importance. I have already discussed this question in part (p. 23), but I wish to return to it here. Experiment 22 shows the results of examining a series of moths from the time of cutting out up to the time when examination usually takes place, and it will be seen that spores were present in greater numbers than developing forms from the very start, so that infection could have been detected on the day of cutting out. It is evident to me that if sufficient spores are not present to make the infection detected on the day after the moth lays its eggs, the infection must be so slight that it will not be likely to cause any harm. It seems better worth while to risk an occasional very mildly infected moth being undetected by beginning the examination early than to " rush " the examination in one or two days when more highly infected moths are even more likely to be overlooked in the hurry. I can find no evidence to show that spore-formation does not take place abundantly in ten days. All this fuss is the result of Mukerji's erroneous ideas about the development of *Nosema*—Hutchinson (1920) evidently accepts as evidence " the experience of seed rearers in Bengal " but that of course is merely the result of Mukerji's teaching. My experience is that in the hot weather and rains—when the moths have to be examined according to this idea in about two days—five days having to elapse after laying before examination is begun —at that time abundant spore-formation takes place, as has been

shown, in four days from date of infection. Further as multiplication of the parasite is rapid at this time, even supposing infection took place only on the day before spinning, by the time the moth cut out and coupled and laid its eggs at least 10 days must have elapsed—the pupal stage is amply long enough for the infection to have shown itself sufficiently for diagnostic purposes. Hutchinson (1920) has shown that one infected meal immediately before spinning may serve to give rise to infected progeny in the next generation, but it is well to note that spores were demonstrated quite easily in the pupæ as well as in the moths.

There is one point in favour of delaying moth examination and that is that the more weakly moths die off, so that a sort of selection of the fittest is brought about. This is a distinct advantage and doubtless helps to keep the stock strong. It is therefore a question if it is worth while abandoning this advantage in order to secure a more leisurely examination, a question that should be decided by the number of examinations that have to be made. It can never be good to hurry examinations unduly.

We may now turn to the method of moth examination to be employed. Much has been written about the " blind "application of Pasteur's method to India. Pasteur's method of seed selection consists in the rejection of all moths heavily enough infected with pebrine to show the spores in a rapid examination—this does not necessarily mean the discovery of every moth containing spores of *Nosema bombycis*, for very mild infections may be discovered only after prolonged searching. The whole point of the method was that heavy infections could be detected by a very rapid examination, and only really heavy infections were dangerous. I believe that this is as true in India as in Europe ; pebrine is not a more serious disease here than in the West—if it were the silk industry would long ago have died out. The routine method advocated by Pasteur consisted in rubbing up in a mortar with a few drops of water the female moth. A drop of the resulting emulsion was examined under a microscope and if no pebrine spores were found in a rapid glance at one or two fields at most, the moth was passed " disease free." The " blind application " of this method in India consisted in taking a moth between finger and thumb in a piece of paper and squeezing some of the hind-gut contents out and examining this—obviously something quite different from Pasteur's recommendation, and a method, which, needless to say, gave deplorable results, for the parasite is a tissue or cell parasite and could not be expected to be found in the lumen of the hind gut except in the case of extremely heavy infections. Besides, the contents of the hind gut are such as to make the detection of pebrine spores

in them very difficult. The futility of this farcical "application" of Pasteur's method hardly required to be exposed, and its lack of success certainly did not imply the failure of Pasteur's method for that had never really been tried.

As the subject of moth examination was rather a delicate one in Bengal in the past I have been unable to trace exactly the history of it, but it would seem that Mukerji's "adaptation" of Pasteur's method held the field until about 1915 when Professor Lefroy and Mr. Hutchinson visited Bengal and began to inquire into the question of disease. Whether this caused the abandonment of the above method of examination or not I do not know, but when I visited the Bengal nurseries in 1920 the paper method was not in use so far as I saw, but in most nurseries the moths were being crushed by mortar and pestle in the fashion recommended by Pasteur. As I have said before, examinations were not being well done, the preparations made while I was watching being far too thick and the crushing of the moths being very imperfect, partly no doubt on account of the softness of the body. It is highly probable that when no one was watching the preparations were even thicker and the crushing still less perfect. But strange to say, imperfect as the examinations seemed to be, the results, as will be seen from the census figures, have been wonderfully good. Now if reasonably good results have been got in a comparatively short space of time by a very imperfect application of Pasteur's method, it speaks very highly for it as a practical scheme In Mysore moth examination is also done by crushing the moth in a mortar, but here the instruments used are of the Italian type and much superior to those used in Bengal. The preparations too were very much better made though even here they were not ideal laboratory "smears." Either potassium or sodium hydroxide in 2 or 5 per cent. solution is used instead of plain water.

A second method of moth examination has been advocated by Hutchinson (1920), and it is in use at Berhampore Central Nursery in Bengal. This is a modification of Pasteur's method for examinations for flacherie, and consists in extracting the gut of the moth with the small amount of surrounding tissues that may cling to it and smearing that in a drop of water on a slide. It is claimed that this method is capable of detecting lighter infections than the other, and personally I much prefer it for ordinary laboratory examinations. Thick, "messy" preparations are not got in this way and the initial cost of mortars and pestles is avoided, the only cost being rough cheap needles in wooden handles. But sericulture is a commercial undertaking and not an academic experiment. Many hundreds of moths have to be examined in a relatively short space

of time, so that speed of working has to be taken into account. The gut extraction method was tried in Mysore and had to be abandoned for commercial reasons—it was found to be much slower than the mortar and pestle method and not sufficiently superior to warrant the expenditure of the extra time. By the crushing method about 600 moths may be examined in a day—this is very fast work and of course claims to detect only heavy infections, but compared with the rate of 1,200 which French examiners claim to have attained it is slow and careful—by the gut extraction method about 240 per day is the maximum number that could be done by one man for any length of time on end. It is true that the number of people employed in the mortar and pestle method is rather large—say, three in addition to the examiner, one to crush the moth, one to make the preparation and the third to wash the utensils—while really only one is required by the extraction method in addition to the examiner, namely, one to wash the needles and remove the papers with the bodies of the moths. Still this extra labour is practically unskilled, children could do it, so that it is cheap. Where small numbers of moths are to be examined the gut extraction method is undoubtedly to be recommended, especially for maintaining the stock in a nursery, but in making a large grainage, I am afraid, it is not a sufficient improvement on the crushing method to warrant its being taken up.

If mortars and pestles are to be employed the Italian system as used in Mysore is undoubtedly the best, and sodium or potassium hydroxide should be used instead of plain water. It is probably necessary for the sake of speed to put four or five preparations on one slide, but if this is done great care should be taken to see that the fluids from the adjacent preparations do not run together. If cover-slips are used, very thin ones are not necessary and they should be small, not more than $\frac{3}{8}$ of an inch square—square are cheaper than round—in this way several preparations can go on one slide without risk of their touching. The microscope should be used upright, not slanting, when wet preparations are being examined. The table and stool—if one is used—should be carefully adjusted for the examiner who uses them, so that he can sit easily without straining his neck—if the seat is too low for the table so that one feels inclined to tilt the microscope, cut a portion of the legs of the table or use a higher stool. Preparations should always be *thin*—only a small drop of fluid is needed and it should not contain large portions of the moth's anatomy. Such small points as these are not so trivial as they seem. It is the little things that worry one and lessen one's speed in microscopic work. "Tips" such as these will suggest themselves to examiners as they work.

The examination must at all costs be careful and honest. That is the great point. Whatever method is used—and the best that can be afforded should be employed—every preparation should be made with the greatest possible care and every examination should be unimpeachable. If a fairly high percentage of pebrine, say, 10 per cent., is found in a lot it should be totally rejected. Efficiency of examinations is a thing that cannot be too strictly insisted on.

In these ways, by disinfection and more efficient examination, the disadvantages attached to the multivoltine worms can be to some extent lessened, but it must be admitted that there are these disadvantages and unless the above means are taken to remove them, at least in part, there will be more danger of infection in India than in a temperate country, first by diseased moths not being detected in a hurried examination and second by any infection which may have been present in a rearing house in one *bund* not having time to die out before the next comes on.

The question of the environment of the silkworm in India is peculiarly difficult. Under this heading may be included the climate, the food supply, the type of rearing house and the rearer himself. There is no doubt that all of these are of the utmost importance in the study of disease. We have already seen that a hot, moist climate such as one gets in Bengal for several months in the year is not favourable to silkworms and is favourable to the propagation of diseases. We have also seen that the bush mulberry used is not so good as tree and that the amount of food given is often insufficient. The type of house used while not so bad as some would like to make out is imperfectly ventilated and lit. All these things tend to make disease more easily spread and more serious in its results—but we have also seen that most of these things are capable of being improved quite easily.

Here may be noted the rearing done from unexamined seed in Pusa and in Berhampore Central Nursery. The seed was examined in March 1921 and not again after that — the cocoons being selected merely by the eye. The rearings at Pusa were done by my head-rearer, a man of the peasant class, and he did all work himself without any supervision. The first *bund* was reared in a *pukka* house but after that rearing was done in a *kutcha* house. Cages infected with pebrine for experimental purposes were kept in the same house, so that no special precautions were taken to prevent disease except that the worms were well fed and carefully and cleanly reared. Practically no disease was got in any of the lots (Appendix IA and experiment 21, lots 4 and 4*a*).

But here we come to the ryot himself. Any improvement will have to come through him and here is the real difficulty. The

rearer, like peasants all the world over, is slow to change and suspicious of new things, and he is as a rule terribly poor. If the benefits to be derived from a change are very great he may be induced to alter, but a series of small improvements do not appeal to him, especially when they entail the taking of a good deal more trouble. He seems, however, to appreciate the benefit of disease-free seed, for in this way he usually gets a full crop instead of losing "2 annas" or more. Other improvements while very necessary are not so showy, and it will need prolonged and good propaganda work to effect any change.

Thus the question of environment is largely the question of the rearer. Within the limits imposed by climate and the type of worm, which is really in many respects dependent on climate too, something can be done to combat pebrine so that it need not be a more serious pest than in other countries, but it will mean harder and more constant work and supervision. The human factor will be very important in India—more so than in more temperate countries. The Indian rearer has been much condemned for his want of care, his want of cleanliness and his ignorance—failings which he evidently shares with sericultural workers in other tropical countries—and from what little I have seen of him I should fancy he would be a very difficult person to convince of the error of any of his ways, but it is useless to approach him looking for faults. His virtues should be sought out and developed. There is great room for and great hope in a really intelligent and sympathetic propaganda in sericulture, and if anything is to be done towards improving the environment of the silkworm and altering those things that tend to make tropical sericulture more difficult and more precarious than that in more temperate lands it will have to be done in this way through the ryot, for after all he ordains to a great extent the conditions under which the worms are to live.

2. CHUNA, CHUNA-KETE OR CHHIT (MUSCARDINE OR CALCINO).

Definition and diagnosis. The Italian name Calcino and the Bengali Chuna-kete are excellent descriptive terms for caterpillars attacked by this disease, referring as they do to the chalky or lime-like appearance of the dead insect. The onset of the disease is as a rule very rapid and relatively little warning is given until one finds dead worms in the litter. Careful observation, however, would show that gradually the worms become sluggish while the beating of the dorsal vessel or heart is said to become faster than normal. A pale rose-pink colour appears, first round the spiracles usually, and later spreads all over the body just before death. The body

becomes limp and " loses its elasticity." The animal ceases to move and rapidly dies. After death the body becomes rigid and mummified—it does not soften and become rotten—and in a few hours it is usually found to be covered with a white efflorescence, so that it looks like a piece of chalk. If the atmosphere is very dry, however, the white appearance may not be got. If the disease appears just before the caterpillars are about to spin they may be able to make their cocoons but the moths will not emerge, and on opening the cocoons the pupæ will be found covered with the white efflorescence. The appearance of the dead insects is so characteristic and patent to the naked eye that no microscopic examination is necessary for the diagnosis of this disease.

History of the disease. On account of the striking appearance of caterpillars infected with muscardine this disease has been known for a very long time. In the treatise of Olivier de Serres on sericulture already referred to, this disease is recognized and in the later writings it is described frequently. Despite the statement by Geoghegan (1880) that muscardine was unknown in Bengal, it is most probable that this disease—like pebrine—is not of recent origin. Mukerji (Watt, 1893) reported in 1888 that muscardine had " always been known in Bengal." As has now been known for nearly a century, muscardine is caused by a parasitic fungus.

History and life-history of the causal organism. In 1835 Bassi demonstrated that the white efflorescence on a caterpillar that had died of muscardine was really due to the spore bearing " fruits " of a fungus or mould, the vegetative portion of which formed a net work of filaments or mycelium which penetrated the body of the infected caterpillar in all directions (Maillot et Lambert, 1906). There are said to be two species of organisms causing this disease—*Botrytis bassiana* and *Botrytis tenella*. In the case of the latter the rosy colour of the caterpillar attacked is deeper, more red.

The disease is transmitted by the spores which are formed on the outside of a diseased caterpillar after its death. The spores are globular in form and somewhat smaller* and of a less refractive index than pebrine spores (Plate VIII, fig. 4). They are said to remain alive for very long periods—for over three years under favourable conditions. It is not necessary for them to be eaten by a caterpillar in order to cause germination, if conditions of moisture and temperature are correct they will germinate on the outside of the body, each

* Some authors say that the spores of muscardine are larger than pebrine spores. The spores I have measured were on the average about 3 μ in diameter while the average for pebrine spores was about 4 μ long by 2 μ wide. All these spores were killed and stained. The muscardine spores look larger on account of their globular shape.

spore pushing out a delicate filament which penetrates into the body of the worm, often through the spiracles. The filaments which gain an entrance into the caterpillar body grow with great rapidity and form a branching " mycelium " which ramifies all through the body of the worm, drawing its nourishment from the living tissues, especially the fat body. The filaments produce club-like swellings or " conidia " which in turn give rise to fresh filaments or mycelia. The body of the caterpillar thus becomes penetrated in all directions with the fungus. The blood becomes scanty in amount and very acid in its reaction, as are all parts of the body including the usually very alkaline gut, and death of the caterpillar or pupa is rapidly brought about. When the caterpillar is dead, the fungus pushes its spore-producing branches through the skin, and the outside of the diseased body thus becomes covered with a felt of white filaments which give rise to countless spores. For the fungus to develop from the spore to the final stage of spore production and the attendant death of the worm attacked takes in Europe, and in India during the cold weather, about ten days, in hot weather in India the course is much more rapid, only about four days being required for the complete cycle.

A worm that has died from muscardine frequently shows some little time after death a very characteristic production of crystals on the outside of the body. These are said to be ammonium magnesium oxalate and are large and prominent.

It is to be noted that muscardine , like pebrine, is a disease found commonly in other insects, especially caterpillars. In other words, *Botrytis*, like *Nosema*, is a common insect parasite and has doubtless been associated with the silkworm since its wild days. It is not a disease which has attacked the caterpillar recently and because of its domestication, although undoubtedly the conditions of domestication have made the disease much more serious than it is among wild insects.

Amount of the disease in India and loss caused by it. As will be seen from Appendix II, the rearers in Bengal attribute considerable loss to muscardine. During the first part of my census examination (Appendix I) no muscardine was found, and in the course of our tours through the silk districts muscardine was at first hardly at all in evidence. In May a very few cases were found in Kashmir. In September, however, my second assistant found a great deal of muscardine in Bengal, and my census results in this *bund* show total losses from this disease. As a certain temperature and humidity combination is required for the propagation of this disease, this seasonal occurrence was to be expected. Muscardine is a disease that is peculiarly dependent on weather

conditions—if they are favourable the disease is rampant; if they are not favourable the disease hardly shows itself. Despite the fact that muscardine was found by me only during the latter part of the rains, there is no doubt that this is one of the most serious pests, at least in Bengal. The rearers usually calculate on losing about $\frac{1}{4}$ of their crop during the September or November *bunds* but it may be much more serious and frequently the whole rearing is lost.

What are the common sources of infection ? As infection is spread only by the spores, an infected caterpillar becomes dangerous to others only after it is dead and covered with the white efflorescence. The spores being on the outside of the dead body they are extraordinarily easily disseminated. A rearing house and all the furniture may thus become very highly charged with spores and so infect subsequent rearings. The wind and the hands and clothing of those attending on the diseased worms are the commonest means of distributing the spores to a distance. The disease is very contagious, and the healthiest caterpillars are as liable to be attacked by it as are the less robust. The worms are said to be more liable to attack during the moulting periods. Muscardine is not transmitted hereditarily, for the worms attacked always die either before spinning or within the cocoon.

Methods of protecting worms from the disease. When muscardine makes its appearance in a rearing house early in the rearing period the possibility of the worms being killed out by the disease is very real, especially in hot humid weather when the fungus develops most rapidly. If the disease does not reveal itself until after the fourth moult some of the caterpillars affected may spin but few may give rise to moths. As soon as a diseased worm is seen in the litter, the litter should be changed as carefully as possible and the old litter burned. The trays and rearing room should be sprayed with 2–5 per cent. formalin, the strongest that can be afforded —1 per cent. while perhaps strong enough for routine disinfection is not deadly enough to cope with an active outbreak. Next day the litter should again be carefully examined and if any dead worms are found they should be removed and burned and the litter again changed. As a routine, sulphur may be burned in the rearing room daily for several days in succession until no more diseased worms are discovered—about an ounce of sulphur mixed with a very little saltpetre is sufficient for 100 cubic yards of space, the room being as tightly shut up as possible. If a formalin spray cannot be used sulphur may be burned instead—one or two pounds of sulphur mixed with a few ounces of saltpetre being burned for every 100 cubic ards of space. Probably such proceedings could not be adopted

by a ryot—it might be too costly or he might object to the trouble. In this case the likelihood of checking the disease is slight. The rearer should be induced to clean the litter frequently. The smoke of green wood is said to be helpful and might be tried, and the house and furniture, especially the trays, should be smeared with cow-dung, mixing the dung if possible with 2 per cent. formalin. Great care should be taken in the disposal of the litter, which should be burned at once. If the attack is bad the clothes and person of those attending the worms should be carefully washed, formalin water being employed if possible. The habit of the ryots in preventing strangers, especially when disease is prevalent, from visiting their rearing rooms is very sound and doubtless helps to prevent the spread of diseases like muscardine and pebrine. If eggs have been kept in rooms in which an outbreak of muscardine has recently occurred or in rooms near which there was an outbreak, it is advisable to wash the eggs in 1 per cent. formalin and then pure water, before they hatch. Good ventilation and an adequate amount of lighting in the rearing house are excellent preventatives.

Do conditions in India make the disease specially dangerous or difficult to check? The high temperature and humidity prevailing in India, especially in Bengal and Assam, for several months in the year make muscardine especially deadly in this country. The conditions are eminently suited for the germination of the spores, and the life-cycle of the fungus at the same time can be completed in three or four days. The careless habits of the Indian rearer and the great difficulty of disinfecting the *kutcha* buildings add to the difficulties attached to checking this disease. That this pest has not been more destructive in the past is doubtless due to the fact that during the rearing periods the conditions are not always suitable for its spread—either the air is not moist enough when the temperature is right or the temperature is too low when the moisture content of the air is favourable. The disease, although a very dangerous one, can be checked if it is caught in time or at least it can be kept from spreading far and wide and from infecting future rearings, but the average rearer does not worry much about little matters like these. "Darkness, stagnant air, dirt, excessive warmth and moisture are the five things that favour mould" (Banks, 1911). All five are abundantly present in most rearers' houses at some time of the year, so that it must be confessed that it seems more luck than good management that keeps the worms from being wiped out more frequently than they are.

In reporting on the outbreak of muscardine which he found in Bengal in September 1921, my second assistant says that no means whatever were being taken to check it. The rearers were genuinely

distressed at the occurrence, but that did not prevent their leaving dead worms lying in the litter or throwing the litter, when cleaning trays, anywhere outside, to help in the spread of the disease. The matter is admittedly difficult, for the ryot cannot afford to spend money on disinfectants—could not the sericultural departments help here? but surely he could change the litter frequently, burning it and any dead worms. If he will not help himself in very simple, though somewhat laborious, ways, little can be done to assist him. It is interesting to note that in this epidemic the better rearers seemed to be suffering less than the more careless ones.

3. KUJI (FLY PEST).

Definition and diagnosis. Damage such as is caused by the silkworm fly to silkworms is not always regarded as "disease" in the strict sense. Doubtless when the parasite is large enough to be seen with the naked eye one is tempted to look upon it as one large animal preying on another and not as a case of disease caused by a parasite. Yet the maggot of the silkworm fly is as true a parasite as the planont of *Nosema bombycis* or the mycelium of *Botrytis bassiana*, and the injury it causes is as much a disease in one case as in the other. We shall, therefore, very briefly consider it here.

Worms attacked by this parasite show a very prominent black wound-scar at the point where the maggot has entered the body of the caterpillar. This wound remains open and forms the entrance to a sort of chamber through which the maggot gets its supply of air. This prominent black scar is so characteristic a feature that it alone is sufficient for diagnostic purposes. One caterpillar may harbour three or even four maggots and still live for some time. As a rule worms are attacked only when they have passed the 3rd or 4th moult. If the destruction of tissue is great the caterpillar will not spin a cocoon, but if no very serious damage is done by the time of spinning, the cocoon will be spun but the moth will never be formed. Further, the cocoons thus got will be useless for reeling as they will probably be pierced by a small hole through which the larva of the parasite makes its way in order to reach the ground to pupate.

History. I have been unable to find any data referring to the silkworm fly in the past—that is to say prior to the latter half of the 19th century. As it is unknown in Europe this is hardly to be wondered at.

Life-history of causal organism. The Bengal silkworm fly—*Ostræa bengalensis* (*Tricolyga bombycis*)—belongs to the Tachinid group of the true two-winged flies or Diptera. It is found only

in Bengal and Assam. In Japan a similar fly is found known as *Ugimya sericaria*, and in China, Siam, etc., another closely related form *Tachina rustica*. Toyama (1906) is inclined to the belief that this last also occurs in India. The naming of all these flies seems doubtful, and I have used the names that are given in the different books and papers without verifying them so that they are possibly not the correct ones. It is considerably larger than a house fly and is prominently striped in black and grey. It is very active on the wing and makes a penetrating buzz as it flies. Copulation is said to take place in the air, and the female produces about 200 eggs. These are laid on caterpillars, being stuck on to the outside of the skin.* The egg is white in colour and though small can be detected by the naked eye. It hatches in about 15 to 20 hours and the maggot, which emerges, eats its way into the tissues of the caterpillar. The maggot lives on the tissues of the silkworm, especially the fat body, and as mentioned above derives its supply of air from the outside through the hole made by the maggot on entering the caterpillar. The larva lives in the caterpillar for about seven days and then proceeds to make its way out of the caterpillar. If the caterpillar has spun in the meantime it cuts through the cocoon and renders it useless for reeling. The maggot makes for the ground and selecting a soft portion buries itself about an inch below the surface and there pupates. The pupal stage lasts about ten or twelve days and then the fly emerges ready to carry on a fresh life-cycle. The times occupied in the various stages depend, of course, on the temperature, etc. According to Cleghorn the pupæ hybernate during the cold weather. For further details of the life-history readers are referred to the papers of Cleghorn, Sasaki and Toyama.

Amount of the disease in India and loss caused by it. It is very difficult to estimate accurately the amount of damage done by this pest. Our census returns show that the fly may be practically absent from some rearings while others show 40 per cent. infection (Appendices IA and IB). A great deal probably depends on the care and skill of the rearer. It must be admitted that on the whole the fly is a very real danger causing a large amount of damage each year, and that, while the whole crop is rarely lost by it, it is quite possible that on occasions as much as about one half of a rearing may be ruined through its ravages. The crawling mass of maggots to be found sometimes under a heap of stored village cocoons is a sight not easily forgotten, and a very striking proof of the prevalence of the fly and the damage it may cause.

* According to Sasaki (1886) the fly in Japan lays its eggs on mulberry leaf, and the caterpillar thus eats the eggs, which hatch out inside the worm. This does not seem to be the case in Bengal.

Methods of protecting worms. The obvious method of protecting the worms is to keep the fly out of the rearing room. But despite the large size of the fly and its characteristic note this is not so easily done. The method employed by the ryot is to keep the rearing room in comparative darkness so that the fly may not see its victims. This is not a desirable measure and it is extremely doubtful if it is very effective—it is stated by Toyama (1906) that the fly loves shade. In the Government nurseries wire-gauze is used for covering the windows and ventilators, and special "fly-killing rooms" are provided at the doors. This seems to be fairly effective as the number of fly-blown cocoons from the nurseries is small, but it is doubtful if the village rearer can afford to use wire-gauze, and if he would take the necessary trouble to ensure success in using it. The practice of rearing only every alternate *bund* seems to be a good one, but it is not rigidly practised, and even if it were it would not stamp the pest out as doubtless the fly can use other hosts—according to Toyama (1906) in Siam the fly is a common parasite of caterpillars other than silkworms. It is possible that wood smoke may help to keep the fly away, and as the damage is done only towards the end of the life of the worm it ought to be possible to keep a smoky fire burning during this time in the entrance to the rearing room. Of course all maggot-infected worms and cocoons should be at once burned or otherwise destroyed. Mukerji (1899) described various methods by which attempts may be made to keep the fly down. The parasitic "midge" described by Cleghorn (1887) might be encouraged and traps for attracting and killing the flies might be devised. It is very possible that the flies are attracted by certain odours, which might be used to lure them to their destruction. It seems to me that there is room for an entomologist to investigate this problem afresh and to try to devise methods for protecting the rearers' houses against the fly. The Government rearing houses, protected by their wire-gauze, are in a fairly satisfactory position—as usual it is the ryot who needs some simple and cheap means of protection. That the fly will ever be exterminated is more than is to be expected, but measures for its control ought to be tried along the lines mentioned above.

In Siam, according to Toyama, the rearers wrap a piece of cotton cloth round each basket of worms, and in China mosquito netting is used on the doors and windows.

4. ROT OR WILT DISEASES.

The diseases with which we have been dealing up to the present are caused by very definite and easily recognized parasites, and

while they may not be understood in all their details—what diseases are ?—they do not present any very baffling problems to the sericulturist. On the other hand the diseases which we now have to study are of rather an obscure origin and there is little real agreement among investigators about them. Several diseases may be grouped under the above heading but there seem to be only two main types, Flacherie and Grasserie—all the other diseases are probably to be referred to one or other of these two well marked groups.

(*a*) *Kalshira* (*Flacherie*).

Definition and diagnosis. The external symptoms of flacherie are well marked. They usually appear only when the worms are full grown and are just about to spin. The worms become sluggish and then motionless. The fæces are very soft—almost liquid—and the anus is soiled by them. Vomiting may be got, the vomit being usually a clear brownish liquid. The motionless bodies, which often hang head-downwards from a twig attached by their anal claspers, rapidly become very soft and discoloured —the blackening usually beginning in the middle of the body in front of the prolegs. Finally, the body becomes all black and putrid and falls into a liquid condition. There is a peculiar sickly odour associated with this disease. If a caterpillar showing the preliminary symptoms of flacherie is dissected, the gut will probably show an amount of undigested leaf in it and what is more characteristic, the peritrophic membrane will be found to be thickened, soft and easily torn, and slightly opaque. The presence of numerous bacteria—diplococci or bacilli—may frequently be demonstrated but their presence does not seem to be by any manner of means so constant in India as in Europe.

History. Such are the typical symptoms of flacherie, and as they are sufficiently striking the disease was recognized by the earlier writers on sericulture and described by them. It is questionable if we know much more about this disease than, say, the Abbé de Sauvages, who wrote in 1763, despite the fact that a considerable amount of work has been done on it. Pasteur (1870) demonstrated the presence of bacteria in cases of flacherie, and since his day the French workers have been wedded to the theory that flacherie is an infectious or at least a contagious disease. Other workers have, since Pasteur's time, busied themselves over this puzzling plague with varying success, and there is now a tendency in some quarters, especially in Italy, to regard the disease as not contagious. The Japanese workers have been particularly energetic in recent years in their researches into flacherie. In India, according to Mukerji (1899), the disease has always been known.

Causal history of the disease. According to Pasteur there were four organisms to be distinguished in cases of flacherie. These are now classified by most French workers into two groups (Maillot et Lambert, 1906), " Ferment en chapelets de grains " and " vibrions." These may be more simply referred to as a streptococcus (*S. bombycis*) and a bacillus. The first is said to be an organism causing fermentation and the second one causing putrefaction. Pasteur's idea was that, owing to certain more or less ill-defined causes, the food in the gut of a caterpillar was not properly digested, and the fermentation organisms which were present on the leaves multiplied and gave rise to flacherie. The disease could also be brought about by feeding caterpillars on the juices from bodies of diseased caterpillars. Further, worms that were produced from a rearing that had suffered from flacherie were believed to have an hereditary tendency to flacherie. Such are still the beliefs of the French school—the disease is highly contagious, it is caused by the organisms described by Pasteur and it is more or less heritable.

The Italian school, as I understand it, while admitting the presence of the bacteria, do not attribute the disease to their action. The disease is caused by some disturbance of the caterpillar's metabolism, and the bacteria flourish in the favourable medium supplied by the already diseased gut—they are a consequence of the disease, not a cause. The disease is not strictly speaking, according to this view, contagious or heritable.

Several Japanese workers have worked on flacherie and, as far as one can judge, their findings point to several bacteria being implicated in the charge of causing flacherie—" flacherie is caused not by any special bacterium but by several which occur commonly on mulberry leaves " (Sawamura, 1905). But of all the bacteria they deal with the most deadly is what they call the " Sotto bacillus " or the " sudden death " bacillus—identified by them as *Bacillus megaterium* De Bary, or a variety of this species, indeed Aoki and Chigasaki (1916) distinguished *Bacillus sotto* as a distinct species having definite cultural characters and especially agglutination reactions. The pathogenicity of this bacillus for silkworms is shown in many experiments, but the peculiar thing about it is that young cultures or broth cultures of the organism did not harm silkworms much under normal conditions—it was only old cultures on agar that caused sudden death.

The Japanese workers have isolated numerous other bacteria from mulberry silkworms or from mulberry leaves, including *Streptococcus bombycis* and *Streptococcus pastorianus* (Sawamura, 1902-03), but those were found not to be pathogenic under ordi-

nary circumstances—only when the temperature was high. Thus their work would seem to show that under normal circumstances the organisms which are supposed to cause flacherie do not cause this disease, but under abnormal conditions certain bacteria seem to bring about the disease.

Most of the work on flacherie has been done on univoltine worms, so that it was necessary to test those different views on the multivoltine worms of India. In the first series of experiments (Experiment 23) caterpillars were fed on juices from caterpillars that had died showing symptoms of flacherie. In all cases the material fed contained large numbers of the organisms typically associated with flacherie. As will be seen, the death rate among these caterpillars was fairly high, but in the majority of cases very few bacteria were recovered although the dead worms showed symptoms very like those described for flacherie in univoltine worms. The impression got was that the growth of bacteria followed upon the serious digestive disturbances induced by feeding the caterpillars on such unsuitable, injurious substances.

An outbreak of flacherie among the Muga worms in Assam afforded an abundant supply of bacteria isolated from typical cases of this disease. There were two organisms present in abundance in the cultures made from the material collected. These I have called Bacillus *A* and Micrococcus *a*. These were fed to Nistari and Chhotapolu worms both in mixed cultures and in pure cultures, and not a single case of flacherie resulted (Experiment 24.)

In Kashmir univoltine worms of French origin were found suffering from typical flacherie. A variety of organisms were isolated from these. All were bacteria that had already been isolated from mulberry leaves, or the air, or healthy caterpillars or diseased Muga worms. Some of these organisms, Bacillus *A*, Bacillus *C* and Micrococcus *a*, were fed to Nistari worms (Experiment 25, lots 9–12). One case of flacherie resulted in the lot fed on Bacillus *A* grown under anaerobic conditions.

Similarly, bacteria isolated from a moth which showed many bacilli on examination, from mulberry leaves and healthy caterpillars were fed to normal caterpillars. In the lot of Nistari worms fed on Bacillus *B* from the moth (lot 6) one caterpillar died showing external symptoms of flacherie, but no bacteria were present in the gut. Among the moths which resulted from these experimental lots a few died shortly after emerging and one failed to emerge—some of them showed bacteria, some did not.

Thus under normal conditions multivoltine worms do not seem to become infected with flacherie to any extent, even when they are fed heavily on bacteria, some of which at least, as we shall see

later, are evidently similar to those that are said to cause flacherie in univoltine worms and which were isolated from undoubted cases of flacherie. It may be said that on the whole under ordinary conditions multivoltine mulberry silkworms, just like univoltine worms, are not readily, indeed hardly at all, infected with flacherie by feeding them with the various bacteria which are supposed to be the cause of the disease. Such evidence as we have does not point to flacherie in India being a highly contagious disease or one associated with any particular organism.

An attempt was then made to try the effect of high temperatures and humidity on worms fed on the various organisms—a repetition of Sawamura's (1902-03) experiments. Unfortunately, the lamp which was used for heating the chamber went wrong* and the temperature of the chamber went up to such a height that the worms all died off. This happened on the day of spinning so that the experiment was really not seriously interfered with—if the organisms fed were going to prove pathogenic the effects should have been shown before spinning. In one case—lot 1, Experiment 26—there was a very obvious onset of flacherie, nine out of fifteen worms dying. These worms were fed on a mixed culture of the three chief organisms isolated from cases of flacherie found in Kashmir. When these organisms were fed separately three out of six worms that had been fed on Bacillus *A* died; two out of six that had been fed on Bacillus *C* died; none that had been fed on Micrococcus *a* died. Bacillus *A* and Micrococcus *a* from Muga worms and Bacillus *B* from mulberry moth were also tried. One caterpillar that had been fed on Micrococcus *a* died, showing small numbers of Bacillus *A* in its gut. Here again I am able to confirm the results of the above-mentioned Japanese worker—high temperature and humidity seem to be favourable conditions for flacherie, and when certain organisms, notably Bacillus *A*, are fed to caterpillars under these conditions flacherie appears to a very considerable extent.

It is to be noted that in the controls in the hot moist chamber there was also considerable mortality, especially in lot 1. Here the worms were put into the chamber before the 3rd moult was begun, and numbers of the worms were unable to go through the moult. When the 4th moult was entered upon all the remaining control caterpillars, as well as those that had survived from the fed lot, died—becoming flaccid and somewhat discoloured but without exhibiting any great growth of bacteria in the gut. In the controls of the other lots—lots 2 to 7—two out of six caterpillars died showing external symptoms of flacherie. Thus, even without the agency of any special bacteria, worms kept in an un-

* This was in the second experiment. The first attempt suffered no disaster.

healthy atmosphere tend to suffer from flacherie. If the guts of caterpillars kept under these conditions be examined, the peritrophic membrane will be found to be somewhat thickened and slightly opaque, and the gut content is distinctly less alkaline than in caterpillars reared under healthier surroundings. Tested by Clark and Lub's indicators (Clark, 1920) a normal gut=pH= 9·8, while the gut of a worm in a hot moist chamber pH=8·4-8·8. This is a clear indication that the damage is being caused by an unfavourable environment—the growth of bacteria doubtless follows on this and intensifies the trouble. The bacteria, however, cannot be called the exciting cause of flacherie. This is undoubtedly due to some disturbance in the surroundings—the bacteria always present on the mulberry leaf are given an opportunity to multiply and the second stage of the disease is brought about, but this stage does not necessarily result until the caterpillar attacked is dead or almost so, for diseased caterpillars showing signs of the external symptoms of flacherie do not always have bacteria in their guts in large numbers. On the other hand, if a batch of worms beginning to show symptoms of flacherie be kept under observation and examined as the time goes on, an increase in the number of bacteria in the gut will usually be noticed as the external symptoms grow more marked.

The bacteria which are associated with flacherie are limited in number, normally two only being common. These are what I have called Bacillus *A* and Micrococcus *a*. Both are frequently found in caterpillars which are obviously suffering from flacherie, but only the first seems to be capable of actually intensifying the disease when fed to worms (Experiment 26). Moths suffering from what is often called flacherie in India—that is to say, the presence of bacteria on examination for seed selection—nearly always show almost pure cultures of a bacillus nearly related to *A* but differing in some details. This I have called Bacillus *B*. A third—Bacillus *C*—is sometimes found in caterpillars suffering from flacherie. It was more common in the univoltine worms in Kashmir. Two other micrococci—labelled *b* and *c* by me—are also found in diseased worms but in small numbers. Perfectly healthy mulberry worms may show any or all of these bacteria, and they are present in abundance, but especially the micrococci, on mulberry leaves. They may also be recovered from the air—the micrococci again being most prominent here. Thus all the bacteria recovered from caterpillars suffering from flacherie are common forms which can be isolated from ordinary mulberry leaves and healthy caterpillars. I am in complete accord with Sawamura (1905) in his finding that " flacherie is caused not by any special bacteria."

The identification of these everyday bacteria is not always very certain as the descriptions given are not satisfactory. Details of the organisms are given in Appendix IV. I have there given what I believe to be the correct names for them, but while the bacilli are probably correctly diagnosed the descriptions of the micrococci to which I have had access are so insufficient that I am not at all satisfied with any names which can be given to them. As will be seen, my Bacillus *A* is probably *B. megaterium* var. *bombycis*, Bacillus *B* is *B. mycoides* and Bacillus *C* is *B. coli*. Four of the micrococci I isolated are perhaps the same as those got by Sasaki (1910) from caterpillars suffering from grasserie, but I cannot quite identify those of Sawamura (1902-03). The details are, however, given in the Appendix mentioned.

The work of Sawamura (1902-03) showed clearly that the most potent exciting causes of flacherie were high temperature, high humidity and bad ventilation—the last being to some extent at least the cause of the first two. I have been able to confirm this view to some extent. Other causes are said in practice to give rise to outbreaks of flacherie, some of these are dirty leaf, wet and fermented leaf, coarse leaf, overfeeding, overcrowding, etc. Attempts were made to bring about flacherie by some of those means without any success. According to Mukerji, Chhotapolu worms are peculiarly liable to flacherie, and for this reason these worms were chosen for these experiments and further these worms were hatched from eggs laid by a highly pebrinised moth. In short, nothing was left undone that might have brought about an onset of the disease. As will be seen from Experiment 27, there was not a single case of flacherie in any of the lots. An attempt was made to see if making the caterpillar gut acid would cause flacherie. Very weak acids had no effect but when the leaf fed to the caterpillars was first soaked in 10 per cent. hydrochloric acid and then fed to the worms, on one occasion all died of flacherie (Experiment 28, lot 4). The worms in this lot showed every symptom of flacherie and cultures made from the gut gave abundant growths of Bacillus *A*. At the same time worms were similarly fed on leaf treated with ammonium hydrate. Five per cent. ammonium hydrate gave two cases of flacherie while 10 per cent. gave no results. Cultures made from the guts of the dead caterpillars in this case gave numerous colonies of Micrococcus *a* and a very few Bacillus *A*. When the experiment with the 10 per cent. hydrochloric acid was repeated no cases of true flacherie were got. The amazing thing in this experiment was that when the caterpillars showed symptoms of disease the gut content was highly alkaline (pH=9·8) despite the fact that the worms were eating strongly acidulated leaf. The gut juices had obviously neutralized the acid

completely. On the other hand the worms that showed symptoms of disease had an acid gut (pH=2·6).

A consideration of the work of the various Japanese workers and of the few experiments which I have made leads one to lay stress on certain points. (1) Under healthy conditions silkworms are not capable of being infected with flacherie by any of the organisms isolated from caterpillars that have died of this disease. (2) The organisms found in the caterpillars suffering from flacherie are common free-living bacteria that are normally always present on mulberry leaves or in the air. (3) The conditions necessary for infection seem to be a high temperature combined with a high humidity. (4) The gut contents of a caterpillar suffering from flacherie are always less alkaline than in a healthy caterpillar. (5) The gut contents of a caterpillar subjected to high temperature and humidity are less alkaline than in a worm under healthy conditions—indeed they are of the same alkalinity as a worm suffering from flacherie—but no bacteria or very few bacteria may be found in these worms.

These facts seem to justify one in coming to certain conclusions. Flacherie is not caused by any one bacterium although certain forms are more commonly got in diseased worms than others, and experiments seem to show that these few bacteria when fed to caterpillars in large numbers and under certain conditions of temperature and humidity tend to produce flacherie very readily. Symptoms exactly corresponding to flacherie are sometimes got by subjecting caterpillars to treatment which lessens the alkalinity of the gut, and in these cases bacteria are frequently not present in the gut. Under these circumstances it cannot be said that flacherie is an infectious disease in the strict meaning of the term.

There is obviously a close connection between decreased alkalinity of the gut and flacherie but it is not at all certain that bacterial growth is the cause of this. Indeed the absence of bacteria in some cases would rather militate against this view. It is supposed that a high temperature and humidity are favourable conditions for bacterial growth, and these, producing certain bye-products, reduce the alkalinity of the gut. But it seems to me that the evidence shows rather that the alkalinity of the gut is lessened by some disturbance in the metabolism of the caterpillar, and that this gives the bacteria normally present on the leaf a medium in which they can multiply very rapidly. The caterpillar in the first instance may be said to suffer from "acid indigestion". In some cases this may even cause death without the intervention of any bacteria; normally, however, a multiplication of bacteria follows upon this lessening of the extreme alkalinity of the gut—fermenta-

tion and putrefaction are set up in the gut and the caterpillar rapidly dies.

The question of the production of toxins by the bacteria which thus multiply is not an easy one to settle. Sawamura (1902-03) was of opinion that no toxin was produced, the damage being caused by such simple products as " ammonia formed by protein decomposition, or of nitrites formed from nitrate contained in the leaves, or of acids produced from the carbohydrates." On the other hand, Aoki and Chigasaki (1916) have shown that *B. megaterium bombycis* produces a toxin which is very fatal to silkworms. These two workers found that a six days old agar culture of the bacillus produced under normal circumstances almost instant death. My experience does not agree with theirs, for none of the bacteria which I isolated from diseased worms produced any effect on normal worms under normal conditions even when the cultures fed were 6 days old or more (Experiments 25 and 26). In this my findings are comparable with Sawamura's. It is possible that Aoki and Chigasaki had a particularly virulent strain of *B. megaterium.*

I have referred to certain cases of " flacherie " in which bacteria were not demonstrated. Probably some writers would not admit that the term is correctly used—the presence of bacteria being necessary for a correct diagnosis. Personally, I believe that this view—that of the French workers—is rather extreme, and I would incline more to the view of the Italian sericulturists that the bacteria are not the exciting cause of the disease but a consequence of an abnormal condition of the gut. This " acid indigestion," as I have called it, may even kill off weakly worms. The symptoms are the same as those for flacherie—vomiting, diarrhœa, thickening of the peritrophic membrane and brown colour of the gut contents. Discolouration of the body may be noted—the brown gut contents serving to account for this at first. After death, of course, there is multiplication of bacteria in the gut and finally putrefaction accompanied by blackening of the body. Worms showing these symptoms are, I believe, correctly diagnosed as suffering from flacherie even if bacteria cannot be demonstrated. While this is so, it must be admitted that the multiplication of bacteria usually got as consequence of this digestive disturbance is so characteristic a feature of the disease, that the presence of numerous bacteria without any of the ordinary symptoms of flacherie would justify one in condemning the worms or moths as suffering from flacherie—it being taken for granted that this multiplication of the bacteria is a clear indication of the metabolic disturbances which we have called " acid indigestion."

Amount of the disease in India. While flacherie is responsible for very heavy losses in European and in Japanese sericultural establishments, it is not a very serious pest among multivoltine worms in India. In Kashmir when univoltine worms are reared, it frequently causes serious loss, but in my tours in India I have rarely seen many cases. In Appendix II it will be seen that there are certain numbers of rearers who complain of flacherie, but on the whole Mukerji's opinion that "in Bengal flacherie is regarded as less injurious than pebrine, muscardine and grasserie" is still correct. In the census results—Appendix I —a large percentage of flacherie will frequently be found. It is doubtful if these figures represent the true state of affairs however. If bacteria are found in a moth on examination it has been put down as flacherie. In many cases, however, the moths have died previous to examination and the bacteria present are those normally found in dead moths—dead moths were frequently examined in order to make records of pebrine as full as possible. The presence of large numbers of bacteria in a living moth may possibly be an indication that the caterpillar had been attacked by flacherie in a mild form and I have accepted that idea, but after all it is flacherie in the worm that is the important thing, and if the death of worms from this cause is not so common as death from other diseases I do not think the rather high percentages found in some of the examinations of moths need cause much alarm. I cannot find any good evidence in favour of regarding flacherie as a heritable disease (Experiment 27 A).

The common sources of infection. As I have tried to show above, flacherie cannot, strictly speaking, be called an infectious disease. The French sericulturists maintain that it is infectious, and the two bacteria which they claim to be the cause of flacherie may persist in a rearing room from one year to the next and so carry on the infection. We have seen, however, that the food of the caterpillar constantly carries these bacteria, so that this must be taken as the common source of the bacterial infection when it is got. It is possible that the dust from an old rearing room may cause flacherie, but this is probably to be explained by the digestion being upset by the dust and the bacteria normally present in the gut getting the opportunity to multiply to an abnormal extent. If, however, the bacteria are regarded as being organisms of infection, we must look upon the food supply as the source of danger rather than upon the dust which may be present in a rearing room from the previous rearing.

Methods of protecting the worms from disease. Even those who allot the most important part in this disease to the

bacteria are quite clear upon the point that some initial disturbance is necessary in the caterpillars before the disease can start. There are many causes which are blamed for this metabolic disturbance, such as careless handling and conservation of the seed, bad food, overcrowding, bad ventilation, etc. To me it seems that the most important factor is the temperature-humidity one. When both temperature and humidity are high then flacherie is to be expected. I would lay the greatest stress on ventilation and proper spacing of the worms. It may, however, be safely assumed that whatever makes for the general health and well-being of the worm will help to prevent flacherie.

To attempt to keep the worms from ingesting the incriminated bacteria would be fruitless. Attempts have been made (Carini) to feed worms on disinfected leaf but the worms so fed showed much more disease than those fed normally. The disinfection of rearing rooms is always to be commended, but I am very doubtful if it is of much value in connection with flacherie. In France, 1 or 2 per cent. formalin is being used for this purpose. In Kashmir the rearing houses are not disinfected at all, and they seem to be no more—if as much —subject to flacherie than in France itself.

Do conditions in India make the disease specially dangerous or difficult to check? Flacherie is not of very great importance in India despite the fact that the climate for several months in the year might be expected to be extremely favourable to its development. The multivoltine worm, as I have shown in my experiments, is evidently very resistant to gut diseases of this type. Possibly many centuries of somewhat searching treatment have eliminated all but the most resistant races. Univoltine worms reared in the plains of Bengal alongside of multivoltine worms, in the same room and under the same conditions, tend to die off completely from flacherie, while the native worms do not suffer at all. If the univoltines are fed on tree mulberry they do not suffer so seriously, and if great care is taken it is quite possible to rear univoltines from European seed. The point is, however, that the multivoltine worm can stand the normal Bengal conditions while the univoltine cannot—we are therefore justified in assuming that one of the chief reasons that flacherie is not very important in India is that the Indian worm is very resistant to this disease. According to Mukerji, "the principal causes of flacherie do not occur in Bengal." These are:—(*a*) "seed getting spoilt"—in the multivoltine worms seed is kept for so short a period that it is not likely to go wrong. (*b*) "Hereditary tendency" —owing to the habit of the Bengal rearers in seeing the worms

spin before buying the cocoons, the seed cocoon seller cannot sell to him without his knowing if the cocoons are from a lot that had shown flacherie, a thing that is quite possible in France. It is very interesting to find that Pasteur remarks in this connection, "Si j' étais éducateur de vers à soie, je ne voudrais jamais élever une graine née de vers que je n' aurais pas observés à maintes reprises, dans les derniers jours de leur vie." Thus the practice of the Bengal rearer in taking cocoons instead of eggs has an eminent authority in support of it—at least in part. (*c*) "Overfeeding of worms"—a fault—if it is one—the Bengal rearer will never fall into. According to Mukerji (1899) flacherie is common in Mysore—at all events more common than in Bengal—and all because they feed their worms more liberally. I can find no confirmation of this. When I was in Mysore I saw no cases of flacherie and I was told that flacherie was not of much importance. In my experimental lots when the feeding was much more generous than in Bengal I never had any flacherie. (*d*) "Wet, sweating, dewy and fermented leaf"—according to Mukerji the Bengal rearer is very particular about this point, preferring that the worms should starve than that they should be fed improperly. Whatever the real reason may be, it seems an undoubted fact that in India the disease does not seem to be particularly dangerous—at all events it is less so than in Europe. Any advances in the improvement of rearing methods will naturally help to check flacherie, but of course, as usual in silkworm diseases, once the disease has appeared little or nothing can be done to check its immediate progress.

(*b*) *Hansa, Tatka or Salpha* (*Gattine or Lucettes*).

There are a number of different names given to a variety of very ill-defined diseases, a few of which are given as a heading. They are all so badly described that it is practically impossible to effect a proper diagnosis. In Bengal the rearers occasionally complain of "salpha"—very rarely of any other trouble of this sort. Salpha, meaning translucent, is evidently the same thing as the "lucettes" or "clairettes" of the French sericulturists. The most characteristic feature in worms attacked by this disease is their translucent appearance due to the almost complete absence of food from the gut and the skin becoming semi-transparent. Worms become greatly emaciated and gradually die, decomposing after death as in flacherie. Bacteria are frequently present in the gut in large numbers, and the peritrophic membrane, just as in flacherie, is thickened and slightly opaque. These are practically the symptoms usually given for gattine—excepting the trans-

lucence so that gattine and lucettes or salpha are practically the same thing. According to Pasteur there are only four silkworm diseases—pebrine, muscardine, flacherie and grasserie—and all these various disorders of the gut are to be referred to varieties of flacherie. They are evidently all brought about in much the same way and the course of the disease leads to the same results—the death and putrefaction of the animal. Gattine is stated to attack the silkworm in its earlier stages—unlike flacherie which usually shows itself after the fourth moult. A peculiar feature is said to be the growth of the hooks on the prolegs, so that these become extremely long and cause the larva to cling strongly to whatever it crawls over (Banks, 1911).

As far as my experience goes, I have never found worms in the young stages attacked, nor have I found the peculiar development of the hooks on the prolegs. Many of the worms kept in the hot moist chamber, however, showed symptoms of what might doubtless be called salpha, but between these and the most typical flacherie cases there were many intermediate forms. The trouble was evidently caused by the same factors, so that I do not feel inclined to admit it as a separate disease. I prefer to adhere to Pasteur's classification of four diseases—all these other " diseases " being merely varieties or combinations of the original four.

(*c*) *Rasa* (*Jaundice or Grasserie*).

Definition and diagnosis. Here again we have to deal with a very well marked disease. It attacks worms as a rule towards the end of the larva life—rarely before the third moult, usually after the fourth. The worms attacked are restless, bloated in appearance and yellow in colour. The skin is frequently shiny and is very easily torn. The blood is turbid instead of clear, and when examined under the microscope it is found to be full of minute polyhedral crystalloids. These bodies may be found in all organs of a diseased caterpillar. They are formed in the nuclei of the cells, chiefly of the fat body, and are the result of degeneration which may be brought on in several ways.

Polyhedral bodies are transparent and brittle, so that they may be crushed by pressing on the cover-slip. They are not very easily stained, but may be coloured with such dyes as carbol-fuchsin or picric acid. They are very resistant to such reagents as acids, weak alkalies, chloroform, ether or alcohol.

An excellent account of these crystalloids and jaundice in general is given by Sasaki (1910), to whose work I am indebted for much information.

History of the disease and its causation. Being a disease marked by very characteristic symptoms, grasserie has long been known to sericulturists, and accounts of it are to be found in the publications of the earlier writers on sericulture. Certain workers, chiefly Bolle and Prowazek have tried to prove that a protozoan parasite was the cause of this disease, but their work has received no confirmation and is not generally accepted. It seems evident that this disease is probably due to some metabolic disturbance, and for this reason I have classified it along with flacherie. Polyhedral bodies—the most characteristic symptom of grasserie—are found in silkworms that have been attacked by the fly parasite or that are suffering from some other disease common to silkworms, such as flacherie or pebrine. If worms are carelessly fed, especially if they are given mature leaf first and tender leaf afterwards, or if after a period of dry weather showers of rain are got, making the leaf very succulent and watery after being very hard and dry—this factor can of course be of much importance only in Bengal where the bush mulberry is cut down every *bund* —if the respiration of the worms is interfered with in any way by closing up the spiracles, if the air in the rearing room is damp and cold and ventilation is defective, then it is said grasserie may be expected. It is not, however, an infectious disease.

Amount of disease in India. While grasserie is of absolutely no importance in Europe it is quite a serious pest in India and in the East generally. As will be seen from Appendix II, the rearers in Bengal almost without exception complain of the ravages of this disease. It is said to be important also in Madagascar, French Indo-China and Japan. In India it occasionally assumes grave proportions and may cause heavy losses in a rearing. As grasserie is a plague found only in caterpillars, it does not appear in our census of cocoons. Further, in my tours in the silk districts I have found only a few isolated cases, so that one might tend to look upon it as relatively unimportant. This must not be done, however. It is probably one of the important destructive factors in Indian sericulture. Rearers estimate that they may lose as a rule about $\frac{1}{4}$ of their crop from this disease.

What are the common causal factors? We have noted above that grasserie is not an infectious disease, but that it is caused by unsuitable food or faulty ventilation. It is also a sequel of certain other silkworm diseases.

Methods of protecting worm froms disease. As grasserie is the result of bad food or insufficient ventilation or some predisposing disease, any improvement in rearing and the general care taken of the worms will help to prevent an outbreak. When the

disease assumes serious proportions, it is then said almost always to be traced to the feeding of very succulent leaf got when a rain storm follows a long period of drought. As Mukerji (1899) points out, under the bush system of mulberry cultivation this is bound to result, but if tree mulberry could be grown and used at such times, the bad effects of very succulent leaf would be avoided to some extent, as tree leaf does not become so rapidly charged with watery sap after rain. Like flacherie this disease is to a very considerable extent the result of climatic conditions, and it is thus impossible to take any very perfect measures to prevent it, as climate cannot as yet be controlled. The occasions on which a very serious outbreak occurs are strictly limited however, and the sporadic cases can to a large extent be prevented by careful rearing.

Do conditions in India make the disease especially dangerous or difficult to check ? The somewhat slovenly, lazy methods of the rearers in India tend to make sporadic grasserie more common and more dangerous than in Europe. The climate is such that "epidemic" grasserie is bound to occur occasionally under the present methods of mulberry growing and even to some extent under the best conditions that one can achieve in India. There is no reason, however, why grasserie should be on the increase as some rearers say (Appendix II, cases 1, 2, 3, etc.)—this is undoubtedly a disease which can to some considerable extent be controlled by care and good management, and as the spreading of the knowledge of better methods takes place decrease in grasserie may be expected.

(*d*) *Lali, Rangi or Kur-Kutte* (*Court*).

The " disease " known as Lali or Court is a typical example of the uncritical attitude of sericulturists, for it is probably not a disease at all but a symptom of some disease. When caterpillars are unable to spin or can spin only a very little silk they pupate in the open, so to say, instead of hidden within a cocoon. The pupa is much shorter than the worm and red-brown in colour—hence the names applied to this condition. When such a state of affairs exists the caterpillar is practically always found to be debilitated from some disease. An odd caterpillar may be found pupating without being able to spin and no obvious cause may be found —it is not an uncommon thing for worms to die sporadically without any definite symptoms of disease, in which cases death is probably due to some previous injury which was undetected at the time, but in all the cases of lali that I have seen pebrine has been responsible for this condition. In bad cases of pebrine the

silk-gland is very heavily attacked (Plate II, fig. 1), so that frequently no silk or very little silk is produced. In the case of the progeny of diseased moths the offspring are often very weakly without showing very much infection themselves. There again silk may not be produced, or if it is, the worm may be too weak to make a cocoon. This so-called " disease " then is nothing but an indication that the worms are greatly debilitated usually—practically always, if there are many cases in a rearing—from the effects of pebrine. Mukerji (1899) mentions that lali is more common in February and March owing to the leaf at the season of the year being " wanting in nutrient properties ", but he points out that " when any considerable loss takes from this cause it should be put down to latent pebrine. Worms reared from seed free from pebrine are seldom affected by rangi."

As will be seen from Appendix II—note especially lots 1, 2, 3—Lali is complained about by the rearers to a slight extent, chiefly by those who rear village seed. If good seed were used the trouble would probably be of even less importance.

III. DISEASES OF THE MUGA SILKWORM (ANTHERAEA ASSAMENSIS).*

As the Muga worm is only partially domesticated, living all its larval life in the open, it might be thought that it would be more free from disease than the completely domesticated, almost helpless, mulberry worm, and to some extent this is true. The parasitic diseases are relatively of little importance. Muscardine seems to be practically unknown, and *Nosema* spores while present cannot be said to be symptomatic of disease to any appreciable extent (Appendix I E). Black spots are said to be found at times on the bodies of muga worms—these worms are then known as "photukia." I have not found any of them either in the worms I have examined in Assam nor yet in those that I reared in Kalimpong. It is, however, possible that these are pebrine spots.† The affected worms are said to be unable to spin cocoons. However this may be, there is no question of pebrine becoming, at present, a disease destructive to muga. As we saw when considering the question of pebrine in the mulberry worm, the parasite, *Nosema bombycis*, is not under natural conditions a deadly one. It is only the disturbance caused by domestication and the unhealthy and insanitary conditions involved that have made it so. The comparatively open life of the muga caterpillar should prevent anything in the shape of an epidemic of pebrine or even occasional loss from that disease being got. For all that, an endeavour should be made to use only examined seed. Seed cannot be too good, and the elimination as far as possible of even a relatively harmless parasite is bound to be beneficial and to help the worms te resist certain other diseases which are undoubtedly serious.

The silkworm fly is a thing that has to be reckoned with, as is an ichneumon fly, but it is of course impossible to protect the worm against these. Given the freedom of well-sized trees, as these worms are, the toll that other insects levy is very heavy—as is the loss caused by insectivorous birds, bats and lizards.

---

* According to the note by J. Henry Watson given by Lefroy (1916) on p. 153 of the Appendix Vol., at least 3 distinct species are being bred in Assam as muga—the eri and the tasar are also said to be mixed. It is possible that more hardy races of muga could be got if these species were separated and bred true instead of being crossed indiscriminately as they seem to be at present.

† Since writing the above I have succeeded in obtaining specimens of muga worms suffering from "photukia" through the kindness of Mr. Dutta of the Assam Sericultural Department, who diagnosed the disease and sent me specimens. It is typical pebrine—the spots being the same as in mulberry worms but I think rather smaller and more regular. Mr. Dutta estimates the loss from this disease at about 3 per cent.

It is, however, only the rot diseases that cause really serious loss to the muga rearer. I was fortunate enough to be able to study an outbreak of flacherie which occurred in the neighbourhood of Titabar in March 1921. The external symptoms were typical. The bright green colour of the worm became dulled and it gradually ceased to feed. The fæces were semi-liquid and many worms were vomiting a brown, clear vomit. The body became very flabby and death ensued. The worms just before death and for some little time afterwards hang downwards from the twigs attached to them by the anal prolegs, gradually turning quite black and sooner or later dropping to the ground, all the organs and tissues dissolved into a putrid black fluid. When the gut of recently attacked caterpillars was examined it was found to contain undigested leaf, or very often a brown, soft, somewhat jelly-like substance. The peritrophic membrane was thickened and somewhat opaque. As a rule the blood in these caterpillars was rather scanty in amount. Strangely enough, bacteria were not found in any numbers under the microscope except when the worm had been some time dead and decomposition had set in. On cultivating gut contents, however, numerous colonies of Bacillus *A* and Micrococcus *a* were got with a very few colonies of Micrococcus *b*. In smears made from the gut of still living worms a small number of micrococci were seen and a very few bacilli.

The two chief bacteria isolated from this outbreak of disease were fed to seven different lots of muga worms on three different occasions (Experiment 29). The results were very confusing. In the first place the controls all showed a certain amount of disease, the symptoms being those of flacherie, and in most of these cases bacteria were got in the gut. The muga worms used in these experiments were all reared inside the laboratory on branches of "mezankuri" (*Litsea citrata*) placed in bottles of water with cotton wool stuffed into the neck of the bottle round the stem to prevent the worms from leaving the branches. For worms accustomed to an open life the conditions were not ideal, and it is surprising that they were as healthy as they turned out to be. The method of rearing was doubtless responsible for the disease among the controls. The gut of a muga worm feeding normally in the open is strongly alkaline (pH=9·8). The worms which I reared in the laboratory were much less alkaline, showing the same hydrogenion concentration as many of the mulberry worms kept in the hot moist chamber (pH=8·6). Furthermore, the controls and the ones that showed obvious symptoms of flacherie were of the same degree of lessened alkalinity. The fed lots then could not be expected to be free from disease; the point to determine was, were they more diseased than the controls? On the whole

they had a much higher mortality, which might be explained by the handling involved in feeding, but the curious fact was that frequently the dead caterpillars showed no organisms in gut although they had all the external symptoms of flacherie. Further, worms fed on the micrococcus frequently showed the bacillus only after death. The organisms were also fed to mulberry caterpillars (Experiment 24). None of the caterpillars thus tried died when fed under normal conditions.

On the whole it is to be concluded that flacherie in the muga worms is very much the same thing as in the mulberry worms. It is not an infectious disease, and it is brought on by some disturbance in the metabolism of the caterpillar. What causes this disturbance in the muga worm is evidently some sudden change in the food. In the early weeks of the year (1921) in which the outbreak I investigated occurred, the weather was very dry and there was but little fresh leaf on the trees when the newly hatched worms were put on them. This seems to have caused a little disease to appear. After the worms were partly grown a considerable amount of rain fell. There was a rush of new succulent leaf, and then the disease began to show itself on all sides. It is not unlikely that the temperature-humidity factor might come in here too but I have no exact data regarding this.

It is practically certain that these outbreaks of disease are caused by climatic variations and vagaries, and for this reason it would be very difficult to prevent rot diseases absolutely, but a great deal can be done to improve matters by developing a suitable type of food-tree—first selecting the best food-trees and then studying the best method of cultivating them. A standard type of tree grown as a cultivated plant and not allowed to go ahead anyhow seems to me the most suitable form to aim at. There is room for much excellent work in evolving this type of food plant and in studying the best methods of cultivating it. Whether the muga rearer would bother to take care of such a tree and prune it and cultivate the ground round it is quite another matter, and that must be decided by the authorities in charge. I am confident that much improvement could be effected by rearing examined seed in a properly managed farm or food-plant plantation. It is possible too that the muga worm would respond to rearing in a cooler, less humid climate than that of the Assam valley. Wild *Antheræa* are found at Kalimpong at an elevation of about 4,000 feet and there they spin the most excellent cocoons. I understand the Sericultural Department in Assam is intending to start a hill station at Shillong. It will take several years to grow the food plants and get the farm properly started, so that

results cannot be hoped for at once, but this is certainly a very important line of investigation and should be thoroughly explored. The ease with which muga worms seem to take flacherie might possibly point to some weakening of natural resistance, and all methods of increasing this resistance should be tried.

The question of attempting to completely domesticate the muga caterpillar is a very interesting one. In Basu's report (1915) it is said that there is no record of this having been done. I have done it on a very small scale in Kalimpong, but I very much doubt if the results so far got justify the continuance of the experiment. The worms I reared, although they spun cocoons, were not particularly healthy, and it must be remembered that any weakness will probably be intensified by the rather unhealthy life indoors, while parasitic diseases, which are at present relatively of little importance, would get an opportunity of flourishing. It seems to me that the muga industry can best be served by an intelligent modification of the methods already in use. If the muga is really a very mixed race, as has been suggested (*See* footnote, p. 80) it would undoubtedly be advisable to try to breed true races. These might be more reliable than the present mixtures. It is a point that should be investigated.

Muga worms are also said to suffer from grasserie—called "phula-rog" in Assam. I have not come across any muga worms suffering from this disease, but from the symptoms given by Basu (1915) there can be no doubt about the diagnosis. On the whole it seems to be of much less importance than flacherie, only 31 cases being given in the appendix on disease in that report as compared with 124 cases of flacherie. In Appendix V of the same report there is a note on grasserie in which the loss is given as 4 per cent. In this appendix too the polyhedral bodies characteristic of grasserie are said to be oval and different from the crystalloids found in cases of grasserie in other silkworms. The disease is also said to be contagious. This last statement is extremely doubtful, and I should also doubt the opinion that the polyhedral bodies of grasserie in muga are different from those in other silkworms affected with grasserie. As I have not seen any cases of grasserie in muga, however, I cannot express an absolutely authoritative opinion. I think it may pretty safely be taken, however, that if not exactly the same as grasserie in other worms it is for all practical purposes similar—brought about by food and temperature conditions and a sequel to other diseases just as in the case of the mulberry worm. It is a disease in the same class as flacherie, not infectious or heritable but directly or indirectly traceable to climatic conditions. Improvements in the rearing of the worms such as have been

touched on above should serve to lessen the frequence and severity of attacks of grasserie as well as of flacherie.

The report on "The Silk Industry of Assam" by Rai Bhupal Chandra Basu Bahadur, which I have had occasion to quote above, contains a large amount of interesting and valuable information on silkworms and their diseases in Assam, and can be confidently recommended to any who wishes to go into the subject of the muga worm more fully.

IV. A NOTE ON DISEASES OF THE ERI WORM (ATTACUS RICINI).

During my investigations, the Eri worm seems to have been singularly free from disease. I received no notice of any diseases having broken out among them, and my enquiries made to the Assam Sericultural Department have always resulted in the information that the worms were in good condition and showing little disease. The eri worm has been studied in some considerable detail by Lefroy and Ghosh (1912) during an extended experiment at Pusa, so that I have felt there was little to be gained by going over the same ground again. In the report published by these workers and in Basu's (1915) report a great deal of useful information will be found. I wish here just to make a few remarks on their findings regarding disease.

Parasitic diseases are got but with the exception of the "fly" pest, which seems to take a heavy toll in Assam, they do not seem to cause the rearers much trouble. Muscardine and pebrine were both got but they are not factors of much importance. I cannot agree, however, with the opinion of Lefroy and Ghosh (1912) that the absence of actual disease (pebrine) makes the examination of moths unnecessary. *Nosema bombycis* is a parasite of the eri worm, and while it may not cause disease it ought to be eliminated as far as possible. In Basu's report it is stated that an experiment in rearing eri worms on a large scale at Kamrup in 1886 by the Deputy Commissioner came to a very untimely end through pebrine. It is possible that the disease was not correctly diagnosed or that pebrine was not the sole cause of the disaster—it would be rather strange if this isolated epidemic of pebrine had occurred and no other similar case was known—but be that as it may, every precaution should be taken, and certainly in rearing seed cocoons in Government establishments only examined seed should be used. It would not be necessary to examine the seed issued to rearers, if the stock were kept sound.

Just as in the case of the muga worm, it is the rot diseases that seem to be of importance in the eri industry and here too it is flacherie that causes the greatest loss—grasserie is said to be relatively unimportant. The flacherie reported from the eri worm would seem to be the same as that from the muga and mulberry worms. In Lefroy and Ghosh's report it is said that the disease was being investigated by the Imperial Agricultural Bacteriologist, but as far as I know nothing was published on the subject. I isolated the bacteria present on castor leaf and in the gut of a

healthy eri caterpillar, and found that Bacillus *A* and Micrococcus *a* were present, the micrococcus being very abundant. Lefroy and Ghosh (1912) are therefore quite wrong in saying "*flacherie as such* does not exist in eri, nor could it." These workers seemed to think that the "Streptococcus" and the "Vibrio" found in mulberry worms were special bacteria and as such could not be present in eri. As Sawamura (1902-03) has shown, the organisms found in cases of flacherie are common free living forms. These occur also on castor leaf and in eri worms. From what I can find out the disease is the same in every way as in the other silkworms. It seems to be caused, too, by unsuitable food, so that in its early stages at all events it is a sort of indigestion and may be avoided to a great degree by care in rearing, but here again we are "up against" climate in the long run, so that the trouble is very difficult to control.

On the whole the eri worm seems to be the hardiest and healthiest silkworm with which we have to deal in India. As I have said, I have been unable to see any cases of disease, but that is far from saying that disease does not exist. The accounts of disease given in the various reports or books on the subject that I have been able to consult, however, all point to this conclusion. Attention, too, may be drawn to the examinations of eri worms given in Appendix I of Basu's report. The examinations of moths made in my census returns—Appendix I H—also show that such diseases as can be detected by that examination are of slight importance.

V. A NOTE ON DISEASES OF THE TASAR SILKWORM (ANTHERAEA MYLITTA).

I have been unable to obtain any exact information about the diseases of Tasar silkworms or to obtain any specimens of the same. Indeed the tasar industry is so imperfectly known and so little organized that it is practically impossible to get any satisfactory information about it. Something is given by Lefroy (1916) in his report but just sufficient to demonstrate the practical impossiblity of doing anything in the matter. This insect belongs to the same genus as the muga worm and has much the same mode of life, it is therefore to be expected that it might suffer from the same diseases. Mukerji (1899) states that these worms suffer from flacherie—he classes the muga worm as a tasar worm and his remarks apply to all known Indian species of *Antheræa* alike. There are several vernacular names of diseases of tasar worms given by Lefroy (1917) in his report (page 189, Appendix). The meaning of only two seems to be clear: in one case the name means a form of cholera, and in the other that the worm turns black. Both might very well be symptoms of flacherie. In the absence of any really reliable information on the subject of the Indian tasar it is impossible to make a satisfactory statement. As the worm is hardly at all domesticated, at best less so than the muga worm, and as it is grown or collected as a rule in the jungle by aboriginal tribes, any inquiry into the diseases of the worm would be of the greatest difficulty and when made would be of little except academic value as practically nothing could be done to control them.

VI. CONCLUSIONS.

It is extremely difficult to make any satisfactory generalizations regarding silkworm diseases in India. As I have already pointed out, there are five distinct diseases to be considered and three genera of moths involved, besides climates and peoples as different as are to be found within the same compass in the world. What may be true in Bengal will not necessarily hold in Mysore —what is correct for mulberry worms is not necessarily so for muga. There are, it seems to me, five important questions that demand answers. 1 What diseases are found in India ? 2. What amount of disease is found and what loss is caused by disease in India ? 3. What are the chief causes of diseases in India ? 4. Can disease be controlled in India ? 5. Has disease caused degeneration of the worms in India ? These questions have all more or less been answered in the preceding pages, but it may be well, before making any recommendations, to summarize these answers shortly.

1. What diseases are found in India ? All known silkworm diseases are found in India. I believe that the different diseases found in one genus of silkworms in India are the same as the different diseases found in the other genera. The parasitic diseases are certainly the same and the rot or wilt diseases seem to me to be exactly alike—the symptoms are the same, the same organisms can be isolated from the diseased worms, and the origin of the diseases is the same.

2. What amount of disease is found and what loss is caused by disease in India ? The amount of disease varies greatly according to the kind of worm, and the season of the year. Mulberry worms are most subject to disease and eri least subject. In mulberry worms the parasitic diseases are the most important, in muga the rot diseases, and in eri probably the fly. At times the whole rearing may be lost from pebrine, muscardine or flacherie. As a rule, in mulberry worms the rearers used to reckon on losing about one *bund* in five, and they may still do so if they use unexamined seed. On the average even with examined seed they frequently lose up to 25 per cent. of a crop—their usual estimate being "1 to 2 annas." It is almost impossible to decide what proportion of this almost constantly recurring loss is due to actual preventible disease and what to bad rearing, underfeeding, overcrowding, etc. In connection with this point it must be remembered that the rearers in their estimate of 1 to 2 annas loss do not count the poor quality of cocoons spun, that is included in my

estimate of 25 per cent. loss. Amount of disease and loss from disease are very nearly the same thing in India—in the case of pebrine and sometimes muscardine the worms may spin cocoons, so that all is not lost but the quality of the cocoons will usually in these cases be very poor. On the whole it may be estimated that in unexamined seed the amount of disease may vary from 100 per cent. to about 25 per cent. The loss from disease may be about the same. In examined seed there may be practically no disease of a preventible sort (pebrine) or at most a very low percentage, but muscardine, grasserie and the fly may take a heavy toll—up to 50 per cent. or even more—and there is a constant loss, at times rising as high as 25 per cent., due to climatic vagaries and bad rearing.

3. What are the chief causes of disease in India? The most deadly silkworm diseases in India are pebrine and muscardine. These are extremely infectious and are caused the one by a protozoon, the other by a fungus. Now, while the spores of these organisms are the actual agents by which these diseases are transmitted, it cannot be denied that, to some extent at least, there are other factors involved. In the forefront of these are to be placed the lack of knowledge and the want of care shown so commonly by the Indian rearer. While sound seed will do much, it will never completely compensate for bad rearing, and if the seed is not of the best careless rearing will make the yield of cocoons infinitely worse than it might have been. I am well aware that poverty is to be blamed for much. The ryot cannot afford to alter his rearing house to secure proper ventilation and he has not the money to spend on disinfection, but he need not overcrowd his worms or underfeed them however poor he may be. Here there is a fundamental failing shown, and one that is not confined to the East, for it is seen in Europe though to a less degree. Unfortunately the Indian market does not seem to demand the best cocoons—it is quantity and not quality that counts—and while that is so, there will be no real inducement to produce a smaller quantity of first grade cocoons rather than a large quantity of poor grade stuff.

The climate, at all events in Bengal and Assam, is another potent factor determining the outbreak and spread of these diseases. High temperatures combined with high humidity are not favourable to silkworm rearing, nor are droughts followed by downpours of rain. These climatic disadvantages are particularly noticeable in connection with the rot diseases, and indeed I am of the opinion that these diseases are more or less directly to be referred to climatic vagaries. But we have also seen that pebrine is more dangerous

in times of the high temperatures and humidity, while muscardine demands such conditions before it can evince itself to any appreciable extent.

Still another, though less obvious, cause is to be found in economic conditions. I have already said that poverty doubtless keeps the ryot from undertaking improvements, but an economic reason such as a big drop in the price of silk may be the ultimate cause of a big outbreak of disease. This, as we have seen, was doubtless the cause for the great amount of disease in Bengal about 1875. While the increased production in Europe about 1845–50 was the reason for the terrible plague of 1850–70. Examples might be multiplied, but perhaps enough has been written to show how many and various are the factors governing the origin of disease.

4. Can disease be controlled in India? There is no doubt that diseases can be fairly efficiently controlled in India, although it is admittedly more difficult in a tropical country than in a temperate country. Pebrine can be kept from becoming epidemic, and if all rearers could be induced to take examined seed it would be almost stamped out. The ravages of muscardine, by adequate ventilation and disinfection of the rearing houses, could be kept greatly in check, and outbreaks could be kept from assuming very serious proportions by careful rearing and by taking immediate precautionary measures whenever the first dead worms were found. The fly can be kept at bay by netting windows and doors or otherwise guarding against its entrance into the rearing rooms. The rot diseases are admittedly more difficult. Much can be done by careful rearing and efficient ventilation, but there will always be elements that cannot be controlled in regard to these diseases, so that complete mastery over them cannot, at all events at present, be expected.

Disease, I have said, can be to a great extent controlled, but only, be it noted, under certain conditions. The practice of using examined seed must be made universal and improvements must be effected in the rearing houses, in the rearing methods and in the cultivation of mulberry. If these conditions are not fulfilled the disease question must always remain uncertain, and frequent and heavy losses will undoubtedly be the lot of the unfortunate rearer.

5. Has disease caused degeneration of the worms in India? Lefroy in his report points out that the Indian worms have not degenerated according to the opinion of several experts. Cocoons produced by diseased worms are usually very inferior, and if disease is always present the cocoons will always be bad. It has been proved in Cambodia that even when breeding from highly

diseased (pebrinised) parents for several generations no real degeneration is got, and my results have confirmed this. Remove the causes of disease and the inferior cocoons will disappear. Of course, it must not be forgotten that, under given climatic conditions, a certain quality of cocoon will be produced for any particular race of worm—that, of course, has nothing to do with disease but is a purely physiological matter.

VII. RECOMMENDATIONS.

We have seen under the various sections on the different diseases the measures that can be taken to cope with them, and it will be recognized that there is no panacea in sericulture. The problems are those of preventative medicine and as such can be solved only by careful and patient work. There are two great points to be aimed at—(1) the production of sound seed, and (2) the improvement of rearing. Both are equally important, and to attain one without attempting the other would give us much less than half the battle. The two should go hand-in-hand.

At present the only source of supply of reasonably disease-free seed is the Government nursery. As we have seen, the quality of the seed produced in these establishments is remarkably good, and they have in the past done a very valuable service to sericulture in India and will, doubtless, continue to do so in the future. I do not think, however, that it is desirable that the operations of the nurseries should be unduly extended. Doubtless, it would never be found profitable to produce all the seed required in any district in Government nurseries, but I am of the opinion that, before extending the nursery system further, an attempt should be made to get the rearers to produce disease-free seed themselves. The reasons for this opinion are simple. While the Government nurseries produce good seed they do not, at present at all events, greatly forward good rearing, and furthermore, as sericulture is eminently a cottage industry, it would be well if seed production could be fostered as such. It would also ultimately probably effect a saving of money to Government if rearers produced disease-free seed themselves.

Personally, I should like to see the day come when rearers produced all the disease-free seed required in any district, but I fear the idea is too idealistic a one for India. An attempt might, however, be made to induce the better sort of rearer to go in for the thorough disinfection of his house, to take his seed from a Government nursery and to be a little more generous in spacing and feeding his worms. The cocoons thus produced could be confidently sold as high grade seed cocoons. If the practice of taking eggs held in the district, the moths on emerging could be examined and their quality thus assured. If, on the other hand, they were purchased as seed cocoons it would be known that they were of a much higher quality than ordinary village stock, and they could thus be sold with a sort of Government guarantee. Indeed, if these rearers could be got to submit to a certain amount of super-

vision, Government might even buy their stock and resell it to the rearers or at all events guarantee the man a good price, for we have seen that examined seed reared in good surroundings picks up little or no disease. The scheme is merely that of the " selected rearer "—a scheme which has already been tried but which has not so far proved a great success. I admit that it is beset with difficulties and opens avenues to dishonesty, but I think it should be tried on a small scale at all events, for I feel that only by encouraging good rearing can we really hope to advance very much. The seed producer is the best and probably the most intelligent rearer, so that by concentrating on him it will be found if any improvement is to be expected. The details of any attempt along this line will, of course, have to be worked out locally, as customs and habits differ so from province to province. The idea is, however, to help and encourage the best type of rearer to produce seed of such a high grade quality that other rearers seeing this may strive to improve themselves too.

It will probably, however, be found a very difficult task to convince any adult rearer that his methods are capable of improvement, so that it may be found most profitable to try to influence the children. In order to do this schools might be attached to certain nurseries where in addition to a little elementary education a good deal of instruction in sericulture might be given. By the employment of models and pictures the life-history of silkworms and the diseases to which they are subject might be made really interesting and impressive, and one or two simple feeding experiments could be made to demonstrate to the dullest the dangers of disease and bad rearing.

Having trained children in schools it would be fatal to allow them to slip back into the bad practices of the villages, so that some means of helping the pupils afterwards should be devised. Here the selected rearer might be useful. While probably he would object to showing his methods to outsiders, he could be held up as an example of what improved methods led to, and if he were getting a higher price for his cocoons and a surer yield, it might be a strong argument for improvement in others. Further, some touring might be done by the Government sericultural officers among the villages round their nurseries and help and advice could thus be given.

I am very doubtful if the village rearers will ever be capable of examining the moths for seed selection microscopically. The work is not difficult but it demands qualities that I do not believe they possess. Besides, the price of microscopes is such that they could not afford to purchase them and Government could certainly

not give them away. It will probably be necessary therefore for all seed examination of this sort to be done by Government examiners who might also be the officers selected to tour in the villages. The attempt made in Bengal to issue microscopes to rearers does not seem to me to have given any promising result. This need not, however, in any way interfere with the production of disease-free seed by the villagers. If they found that the microscopic examination of seed was a thing to be desired, they might bring their moths to an examination room in a nursery or a member of the nursery staff might go to the villages to examine the seed.

If the ryot is to make any advance in sericulture, he must spend money on disinfectants, on renewing the stands and trays after a big outbreak of disease, on altering his house, where possible, to secure better ventilation. He cannot afford to spend this money in most cases—indeed he does not have it to spend. It will therefore be necessary to arrange to supply the money somehow-or-other. Probably, it could be obtained through co-operative banks, but found it will have to be before progress can be made. Another point may be noted here. I have been told that village rearers would sometimes prefer to have Government nursery seed in Bengal, as they recognize that it gives a better crop, but that they cannot get it advanced to them by Government and so buy from the village cocoon seller, who will give the cocoons and take payment in the future. Here again a co-operative society would be of real assistance.

The Government nurseries will, of course, continue to distribute disease-free seed. It will be impossible for them to slacken any efforts in this direction for a long time, if ever. It is perfectly possible that it may be found impossible to get the village rearers to improve sufficiently to satisfy Government requirements, in the event of which it would probably be found advisable to extend the Government nursery system. There are two points to be noted in this matter—first, Government seed must be absolutely the best in the district, and second, Government must not undersell the local seed-producer and so take his trade away from him. Government seed should be worth the top price and it should take it. By keeping up a high standard the seed-producers will be induced to produce the best article they can in order to compete with the nursery stock, and any attempt on the part of Government to undersell them would be fatal to the best interests of sericulture. Not only would underselling probably kill out the village seed-producer—the man we most want to keep going, for he is the best rearer—but if the rearers got cheap seed they would

very likely not appreciate it however good it was—they would rear it carelessly because it was cheap. And again the latter state would be worse than the former.

The Government nurseries should be run as nearly as possible in a way that the village rearer could emulate. For this reason I am opposed to any elaborate fittings or expensive buildings. A certain amount of experimental work should, however, be done in some of the nurseries, but that should be of quite a simple nature. The different varieties of food plants should be tested and the best selected; various methods of cultivating these, including manuring and pruning, should be tried; the optimum amount of food, space and light in the rearing house should be determined; improvements in rearing-house furniture should be tested and new pieces and methods devised.

Another point that will have to be considered will be the provision of stock for the nurseries themselves. Probably, it will be necessary for one nursery to keep the stock going during the whole year for all the other nurseries. In any case this stock should be of the very highest grade. It ought to be selected from the best cocoons in all the nurseries so that in-breeding is avoided. It should be carefully watched during the caterpillar stage for a great deal about the health of the race can be found out in this way. And finally the microscopic examination of it should be of the most careful sort—the gut extraction method as advocated by Mr. Hutchinson should certainly be used for the selection of the seed in this case.

It is essential that the officers in the sericultural departments should be in touch with modern advances in sericulture, and for this it is necessary that they should have access to the literature of sericulture both past and present day. This literature is of a very considerable volume and difficult to get—practically none of it is in India. It would be impossible for any one sericultural department to get it all. Perhaps, here the Imperial Government might help. There is already a nucleus of such a library in Pusa; this might be added to and the provinces encouraged to borrow books from it. A small catalogue of these works might be prepared, containing not only text-books but references to papers in various journals. In this way there would be a method whereby all who were interested could get access to the literature.

It is also very desirable that sericultural officers should see how things are done in other provinces and countries. For this reason they should be encouraged to visit other sericultural organizations, both in India and especially in Madagascar, French Indo-China and

Japan. Perhaps, scholarships might be given to assist those desirous of widening their outlook and increasing their knowledge.

The question of centralization. The idea of an Indian Silk Institute with a Central Seed Supply Station is extraordinarily attractive, and one which I would fain declare myself in favour of, but the more I see of Indian sericulture the less feasible the scheme appears. I am not in a position to pass any opinion on the commercial side of the question —it might possibly be advantageous to have a central organization connected with dyeing, weaving, etc.—but at the outset I must declare emphatically against a central seed supply station. No one central station could ever supply efficiently all India with seed. Of course, such a supply could not be for the ryots—that would be an absolute impossibility —it would be a source of seed for the different provincial sericultural departments to draw on, but even so the conflicting interests of the various provinces and districts concerned, the great variety of seed that would have to be kept going, the difficulties in arranging adequate supplies to satisfy the demands from widely separated parts of India at the correct times, the practical impossibility of selecting any place for such a station that would be suitable for all varieties of worms and within easy reach of any part of India, are only a few of the objections that rise in one's mind when the idea is considered. In any case it is obvious that a single station would be useless—it would be folly to have all one's eggs in one basket. Some unforeseen accident might destroy a large part of a crop, so that at least two stations would be required, some distance apart, to lessen the danger from this possibility. And when two stations, why not more? Each district would doubtless want one.

And here we come to the real fundamental objection to centralization. India is too vast a country—its diversities of conditions are too great—its requirements too varied. A central seed supply station to be adequate would have to have a section devoted to the demands of each silk district, and for such a section to be really successful it ought to be *in* that district. There is no reason whatsoever why a central station should be called into being to supply disease-free seed. That can be produced in each district for its own use probably much better and cheaper than in an all-India concern.

Owing to the present state of public finances I believe that only the most urgent expenditure should be undertaken. The important thing to do at present is to attempt to eliminate disease as much as possible and to improve rearing. Until this is done or until money is more plentiful, it is most unprofitable to spend

anything on such work as hybridizing. The results up to the present have not justified any further expenditure until ample funds are available, and besides the best hybrid would give poor results if disease were still rampant and rearing bad. I am not opposed to hybridization experiments, but to conduct them adequately is expensive and to do such work inefficiently is futile. All available money should be used to try to improve sericulture in the villages.

I have not considered the question of legislation in connection with silkworm diseases, as I do not believe that anything of value could be enforced. If rearers could be obliged by law to use examined seed, to disinfect their houses and adopt better rearing methods the question of disease would be easily solved; but that cannot be done—it must all be left to the persuasive powers of the sericultural departments.

Ultimately, however, we must come back to the real determining factor in the question of disease in silkworms—the human one. It is a question of men. I believe that the future of sericulture in a very real way lies in the hands of the officers in the sericultural departments. They should be natives of the province in which they work, so that they may be able to converse with the ryots in their own tongue and to understand their characters, customs and prejudices. They should be men of energy, common sense, integrity, sympathy and understanding, rather than trained scientists—there is not a great deal of science necessary for successful sericulture; diseases can be controlled only by persuading the ryot of the error of his ways and helping him to mend them. To attain this end the officers must gain the confidence of the rearers first and foremost, and to do this will demand the type of man I have sketched. The success of the work will depend largely on the officers.

But the ryot will have to play his part. That the peasant of India is willing to adopt new things if they afford sufficient inducement, the substitution of the old wooden sugar mills by the newer iron mills practically all over India would tend to show. But the inducement must be large. Will he be ready to take more trouble, to expend more energy in obtaining only small increases in his gains? Many hard things have been said about the Indian ryot and his methods, a few of which I have quoted; I wish to cite here two opinions both expressed by Indians. The first is Mukerji's: "It is, however, vain to expect that silk-rearers generally will abandon their present methods in preference for others more rational, when they will come to understand the nature of these diseases.....Instead of asking them to alter their ways it is much easier to organize a method of circulation of healthy seed and

effect improvement of the silk-industry in that way." The second is Basu's: "The rearing of silkworms in Assam is surrounded by an amount of prejudice and superstition which to an outsider seems incredible and which may prove a serious obstacle to any improvement that we may try to introduce..... It is true that the Assamese ryot is, as a rule, too lazy and at the same time too well off to exert himself for improving his lot in life." If these opinions are correct the future of sericulture in Bengal and Assam is not very bright. In other parts of India the human material may be better—more susceptible to improvement. I am, however, optimistic enough to believe that even Bengal and Assam are not so hopelessly bad. Given time, improvement may be possible but the task will admittedly be a difficult one.

To my mind the problem of disease in silkworms in India is not primarily one of caterpillar or climate, it is one of men. If the right men can be got to fill the posts in the sericultural departments and if the ryot shows himself to be capable of progress, then the silk industry can be more or less freed from the burden of losses from disease. If India cannot supply those fit to lead and advise and if the peasant cannot change his ways, then no genuine improvement can be expected.

There is no panacea in sericulture. There is no straight road to success. Disease can be conquered only by honest and careful seed selection coupled with the adoption of modern means of disinfecting and rearing. If the ryot with adequate Government help cannot be induced to adopt these simple measures his state is indeed parlous. The matter lies in the hands of the people of this country. If they fail, it will be through the defect of their own character—if they succeed it will be to their own credit.

VIII. SUMMARY.

1. All recognized silkworm diseases are found in India.

2. These diseases are the same in India as in other parts of the world.

3. The diseases of mulberry, muga and eri worms are the same.

4. Pebrine is of importance only in mulberry worms. In village stock it is still rampant, but in stock from examined seed, such as is supplied by Government nurseries, it is not serious. Losses from pebrine are still heavy, however, as the large majority of rearers use unexamined seed.

5. Muscardine is also almost confined to mulberry worms, and it is a most serious pest. Although the procedure for checking attacks is well known, no steps seem to be taken when an epidemic breaks out and whole rearings are frequently lost.

6. The "fly" levies a heavy toll on all silkworms in Bengal and Assam. The measures for checking it are somewhat costly and little is done in this direction.

7. Flacherie is not of great importance as a rule in mulberry worms, except among univoltine ones, probably causing less loss than any other disease. It occasionally causes serious loss among muga and eri worms. As the cause of this disease is mainly climatic it will be very difficult to control efficiently although good rearing and healthy surroundings will do much.

8. Grasserie is said to cause considerable loss in India to all silkworms, but it has not been much in evidence during the period of these investigations. It is frequently a sequel to some other disease but may also be caused by climatic vagaries.

9. While conditions in India are such as to make the control of disease considerably more difficult than in temperate countries, there is no reason why diseases should not be controlled, and where efficient sericultural departments are in existence this is being done with a considerable degree of success.

10. The disease problems in Indian sericulture are ones of preventative medicine and as such they will depend largely on the human elements concerned.

11. The crux of the whole question is the "ryot." If improvement is to be effected the village rearer will have to be instructed in the causes of disease and induced to go in for improved methods of rearing.

12. In order to do this the provincial sericultural departments will require to be recruited from the best type of man available,

and their hands will have to be strengthened in every possible way by Government.

13. The industry is eminently a cottage one and the rearers should be encouraged to go in for the production of disease-free seed themselves. The extension of the Government nursery policy would certainly help to give the rearer better seed but it would do no more. It would leave him actually but little better off than he is at present. Unless the rearer can be persuaded to improve himself practically no real control of diseases can be expected.

14. Financial assistance of some sort is essential, for the average rearer is too poor to afford "improvements."

15. The most important work of the Government sericultural departments should thus be instruction and supervision.

16. The policy of supervised rearers should be given a fair trial.

17. The policy of sericultural schools attached to Government nurseries is highly to be commended. Probably only through the children of rearers can progress be expected.

18. In addition to propaganda work the sericultural departments should conduct experiments in sericultural practice. Especially to be commended are attempts at improving the food plants in every possible way. The introduction of tree-mulberry should be seriously attempted.

19. In order that the various sericultural departments may know what is being done in all sericultural countries an adequate library of sericultural literature should be got together—and it should be used. Further, officers in these departments should be encouraged to visit foreign silk districts both in India and in other countries.

20. Sound seed is essential, but the best seed will give bad results if carelessly reared in unsuitable houses, consequently no scheme for supplying seed can be considered satisfactory that does not attempt at the same time to improve the rearing. Until such elementary principles as these are established it is a waste of time and money to attempt to introduce new races of worm.

21. In the production of sound seed no central seed supply station is required and indeed is probably not a feasible project under any circumstances.

22. Improvements are to be looked for from the work of the provincial sericultural departments working among the villages, but they must be given the necessary time to achieve results and sufficient encouragement to stimulate them in an arduous undertaking.

APPENDIX I, A.

Census of disease in Nistari race of mulberry silkworm.

Lot	Race	Origin of seed	When reared	Where reared	Number of specimens examined	Percentage of pebrine	Percentage of fly-blown cocoons	Percentage of flacherie	Percentage of muscardine	Remarks.
1	Nistari .	Nursery examined seed .	March-April 1921	Village, Murshidabad District, Bengal.	100	3	nil	33	nil	
2	Do. .	Pusa seed examined, 1st generation.	Do. .	Berhampore Central Nursery, Bengal.	30	nil	nil	nil	nil	
3	Do. .	Pusa seed unexamined, 1st generation.	May-June 1921 .	Do. .	181	nil	On an average 1·7	44·7	nil	
4	Do. .	Pusa seed examined, 2nd generation.	Do. .	Do. .	131	0·7	On an average 5·6	33·5	nil	
5	Do. .	Nursery examined seed .	Do. .	Piasbari Nursery, Malda District, Bengal.	100	nil	0·7	5	nil	
6	Do. .	Ditto .	Do. .	Village, Malda District, Bengal.	105	nil	34·8	48·5	nil	
7	Do. .	Ditto .	Do. .	Do. .	85	nil	44	44·7	nil	
8	Do. .	Kalimpong seed examined .	Do. .	Village, Murshidabad District, Bengal.	124	nil	2·95	15·3	nil	
9	Do. .	Ditto .	Do. .	Do. .	130	nil	2·2	10	nil	
10	Do. .	Pusa seed examined, 3rd generation.	June-July 1921 .	Berhampore Central Nursery, Bengal.	154	nil	Figure not noted.	24	nil	
11	Do. .	Pusa seed unexamined, 2nd generation.	Do. .	Do. .	153	0·6		24	nil	
12	Do. .	Kalimpong seed examined .	July 1921 .	Village, Murshidabad District, Bengal.	500	21	4·36	5·2	nil	
13	Do. .	Ditto .	Do. .	Do. .	400	0·75	1·69	6·25	nil	
14	Do. .	Ditto .	Do. .	Do. .	400	1·5	11·53	8·25	nil	
15	Do. .	Ditto .	Do. .	Do. .	500	18·8	0·66	4·2	About 6	
16	Do. .	Ditto .	Do. .	Berhampore Central Nursery.	335	0·88	0·86	21	nil	

Census of disease in Nistari race of mulberry silkworm—concld.

Lot	Race	Origin of seed	When reared	Where reared	Number of specimens examined	Percentage of pebrine	Percentage of fly-blown cocoons	Percentage of flacherie	Percentage of muscardine	Remarks
17	Nistari	Village unexamined seed	July 1921	Village, Murshidabad District, Bengal.	100	97	nil	37	nil	
18	Do.	Ditto	Do.	Do.	100	15	2	21	nil	
19	Do.	Ditto	Do.	Do.	100	62	nil	13	nil	
20	Do.	Ditto	Do.	Do.	100	1	nil	1	95	
21	Do.	Pusa; examined, 4th generation.	July-August 1921	Central Nursery, Berhampore, Bengal.	200	nil	0·08	18	nil	Damage by *Dermestes vullpinus* beetle 4·02%.
22	Do.	Pusa, unexamined, 4th generation.	Do.	Do.	200	nil	0·09	2	nil	Damage by *Dermestes vullpinus* beetle 6·16%.
23	Do.	Nursery, examined	November-December.	Sompara Nursery, Murshidabad.	104	nil	nil	nil	nil	
24	Do.	Ditto	Do.	Chandanpur Nursery, Murshidabad.	246	4·4	nil	nil	nil	
25	Do.	Kalimpong, examined	August-September	Village, Murshidabad District.	Progeny of 5 layings.	..	..	..	100	All the worms died of muscardine before spinning. No examination for pebrine, etc., could be done.
26	Do.	Ditto	Do.	Do.	234	..	64	..	100	Almost all the worms died of muscardine before spinning, No examination for pebrine, etc., could be done.
27	Do.	Ditto	Do.	Do.	125	..	85	..	100	Ditto.
28	Do.	Ditto	Do.	Do.	Progeny of 5 layings.	..	..	..	100	All the worms died of muscardine at the second stage.
29	Do.	Ditto	Do.	Central Nursery, Berhampore.	200	2·5	6·0	nil	nil	In addition to the damage by fly 2% of the cocoons were damaged by *Dermestes* beetle.
30	Do.	Village, unexamined	Do.	Village, Malda District.	100	83	..	..	..	

APPENDIX I, B.

Census of disease in Chhotapolu race of mulberry silkworm.

Lot	Race	Origin of seed	When reared	Where reared	Number of specimens examined	Percentage of pebrine	Percentage of fly-blown cocoons	Percentage of flacherie	Percentage of muscardine	Remarks
1	Chhotapolu	Village seed unexamined	March-April 1921	Village, Murshidabad District, Bengal.	100	100	Average % fly-blown cocoons was 6·3%.	37	..	
2	Do.	Ditto	Do.	Do.	100	99	Do.	9	..	
3	Do.	Ditto	Do.	Do.	100	100	Do.	17·5	..	
4	Do.	Ditto	April-May 1921	Village, District ?, Bengal.	150	100	2·7	12	..	
5	Do.	Nursery, examined	May-June 1921	Government Farm, Darjeeling District.	181	nil	..	..	..	

APPENDIX I, C.

Census of disease in hybrid races, univoltine race and Burma race of mulberry silkworms.

Lot	Race	Origin of seed	When reared	Where reared	Number of specimens examined	Percentage of pebrine	Percentage of fly-blown cocoons	Per centage of flacherie	Percentage of muscardine	Remarks
1	Hybrid	Calcutta	April-May 1921	Calcutta	29	90	..	..	..	
2	Do.	Do.	Do.	Do.	30	63	..	..	..	
3	Do.	Do.	Do.	Do.	50	48	..	6	..	
4	Do.	Do.	Do.	Do.	50	24	..	6	..	
5	Do.	Do.	Do.	Do.	32	56	..	12	..	
6	Univoltine	Kashmir village, examined	Do.	Village, Kashmir	84	nil	..	3	..	
7	Burma race	Village, unexamined	July 1921	Village, Prome District, Burma.	100	100	..	75	..	

APPENDIX I, D.

Percentage of pebrine in control lots of carefully examined seed.

Lot	Race	Origin of seed	When reared	Where reared	Number of specimens examined	Percentage of pebrine	Remarks
1	Nistari	Pusa, examined	May-June	Pusa	75	nil	
2	Ditto	Ditto	June-July	Do.	416	nil	
3	Crossbred Nistari-Mysore race	Ditto	Do.	Do.	161	nil	
4	Crossbred Nistari-Madagascar	Nursery, examined	Do.	Do.	76	1.3	
5	Nistari	Pusa, examined	July-August	Do.	325	nil	
6	Crossbred Nistari-Madagascar	Ditto	Do.	Do.	50	nil	
7	Nistari	Ditto	August-September	Do.	267	nil	
8	Crossbred Nistari-Madagascar	Ditto	Do.	Do.	30	nil	
9	Nistari	Ditto	October-November	Do.	125	nil	
10	Crossbred Nistari-Madagascar	Ditto	Do.	Do.	25	nil	
11	Nistari	Ditto	November-December.	Do.	243	nil	
12	Ditto	Ditto	January-March	Do.	230	nil	

APPENDIX I, E.

Census of disease in Muga worms.

Lot	Race	Origin of seed	When reared	Where reared	Number of specimens examined	Percentage of pebrine	Percentage of fly-blown cocoons	Percentage of flacherie	Percentage of muscardine	Remarks
1	Muga .	Nursery unexamined . .	April . . .	Titahar Nursery .	66	10·6	nil	3	nil	
2	Wild Muga	Collected in Kalimpong .	May . . .	Wild in jungle .	1	The worm was pebrinised.	nil	nil	nil	
3	Do. .	Ditto	September . .	Do. .	2	50·0	nil	nil	nil	
4	Wild Antheræa, ? species.	Ditto	Do. . .	Do. .	2	50·0	nil	nil	nil	
5	Wild Muga	Ditto .	Do. . .	Do. .	3	nil	nil	nil	nil	In a wild muga cocoon the pupa inside was attacked by a small fly, very much like the house fly, and there were a large number of the adult and pupæ of the parasite present breeding.

APPENDIX I, F.

Comparative figures of findings of diseases in different races of silkworms reared at Berhampore Central Nursery.

Name of race	Rearing period	Percentage of pebrine	Percentage of flacherie
No. 1 A, 36th generation	March-April 1919	7·38	27·27
No. 4 A, 36th generation	Ditto	4·63	28·11
J 303 A, 69th generation	Ditto	7·11	14·26
Nistari	Ditto	9·17	18·3
No. 1 A, 37th generation	April-May 1919	5·3	39·18
No. 4 A, 37th generation	Ditto	6·2	15·17
J 303 A, 70th generation	Ditto	15·9	33·18
Nistari	Ditto	9·4	33·33
Chhotapolu	Ditto	5·76	36·53
No. 1 A, 38th generation	June 1919	5·1	56·6
No. 4 A, 38th generation	Ditto	6·3	52·9
J 303, 71st generation	Ditto	7·28	52·84
Nistari	Ditto	7·5	54·4
Chhotapolu	Ditto	8·5	56·7
No. 1, 39th generation	July 1919	7·18	33·43
No. 4, 39th generation	Ditto	8·78	49·65
J 303, 72nd generation	Ditto	7·73	44·88
Nistari	Ditto	6·56	44·94
Chhotapolu	Ditto	11·31	35·16
No. 1, 40th generation	August 1919	10·59	40·82
No. 4 A, 40th generation	Ditto	10·96	47·55
J 303, 73rd generation	Ditto	14	35·2
Nistari	Ditto	23·84	29·54
No. 1, 42nd generation	October-December 1919	5·2	52·7
No. 4 A, 42nd generation	Ditto	10·18	48·96
J 303, 75th generation	Ditto	10·12	47·57
Nistari	Ditto	11·45	48·19

Comparative figures of findings of diseases in different races of silkworms reared at Berhampore Central Nursery—concld.

Name of race	Rearing period	Percentage of pebrine	Percentage of flacherie
No. 1 A, 44th generation . .	March-April 1920 .	6·0	49·25
No. 4 A, 44th generation . .	Ditto . . .	5·0	45·0
Nistari	Ditto . . .	13·93	30·31
J 303, 77th generation . . .	Ditto . . .	6·88	34·48
No. 1 A, 45th generation . .	May 1920 . . .	5·0	38·53
No. 4 A, 45th generation . .	Ditto . . .	5·44	43·12
J 303, 78th generation . . .	Ditto . . .	6·33	23·23
Nistari	Ditto . . .	5·54	39·13
Chhotapolu	Ditto . . .	5·84	46·97
No. 1 A, 47th generation . .	June and August 1920	4·85	25·74
No. 4 A, 47th generation . .	Ditto . .	4·68	22·39
J 303, 79th generation . . .	Ditto . .	7·33	28·44
Nistari	Ditto . .	7·0	34·11
Chhotapolu	Ditto . .	5·6	34·4

APPENDIX I, G.

Extracts from Mysore Farm Grainage Register from July 1920 to end of June 1921.

Month and year	Locality and quantity	Percentage of pebrine or percentage of diseased	Remarks
1920			
July	15,000—Kunigal Farm	7·4	
August	(1) 16,000—Panchama Rearings, Mysore	3·5 Peb. 6·8 Fl.	Reared by beginners and hence high percentage of Fl.
	(2) 10,000—Santhebachahalli Ryot	6·1 Peb.	New area. Departmental supervision.
	(3) 6,000—Mysore Farm	4·4	
September	(1) 500—Ryot of Maddur	8·1	Seed supply, local ryot's.
	(2) 15,400—Kunigal Farm	12·3	Seed got from ryot.
October	(1) 20,000—Bidadi Ryots	7·2	Under departmental supervision.
	(2) 10,000— Ditto	6·25	
November	(1) 27,000—Kunigal Farm	5	
	(2) 21,000—Nagainayala Ryot	5	Departmental supervision.
	(3) 20,000—Bidadi Ryot	2·3	Ditto.
December	(1) 5,000—Hol Dodedehi Ryot	2·6 Peb. 6·0 Fl.	New area—hence percentage of Fl. high.
	(2) 9,900—Bettahalli	3·5	Under Mysore Farm supervision.
1921			
January	11,100—Mylanpatna Ryot	3·4	Departmental supervision.
January-February	(1) 11,635—Mysore Panchama Rearings	2·4 Peb. 6·2 Fl.	Mysore Farm reared shows the least percentage out of the three regarding percentage of rejections.
	(2) 12,000—Mysore Farm	2·12 Peb. 3·23 Fl.	
	(3) 14,635—T. Nasipur Farm	3·4 Peb. 4·0 Fl.	
February	(1) 6,720—Bettahalli Ryot	5·8	Under Mysore Farm supervision.
	(2) 12,840—Mysore Farm	5·8	
	(3) 10,700—Kikkeri Ryot	3	New areas under departmental supervision.
	(4) 91,000—Mylanpatna Ryot	3	
March	(1) 18,100—Maddur Ryot	4·2	Farm supervised under departmental supervision.
	(2) 3,800—Kikkeri Ryot	3·8	
	(3) 9,000—Mysore Farm	2	
April	(1) Kunigal Ryots	14	Not under departmental supervision.
May	(1) 40,000—Kunigal Ryots	8·2	Departmental supervision.
	(2) 25,000—Bidadi Ryots	3·6	
	(3) 40,000—Kunigal Ryots	3·9	
June	(1) 40,500—Bidadi Ryot	6·8	
	(2) 9,900—Kikkeri Ryot	8·6	
	(3) 1,980—Coonoor	6·2	New rearing, Madras Government.

APPENDIX I, H.

Census of disease in Eri silkworms.

Race	Origin of seed	When reared	Where reared	Number of specimens examined	Percentage of pebrine	Percentage of fly-blown cocoons	Percentage of flacherie	Percentage of muscardine
Eri . .	Partially examined seed	August .	Kalimpong . .	10	10	nil	40	nil
Wild eri .	Some eggs of wild eri were got by chance on Mezankuri leaf.	July . .	Reared in laboratory at Kalimpong.	13	nil	nil	nil	nil
Eri . .	Unexamined village seed	August .	Village Titabar, Assam.	100	nil	nil	nil	nil

APPENDIX II, A.

Rearer	Name of village	What diseases are found ?	Are diseases getting more common ? If so, what diseases ?	How much loss is caused by different diseases ?	Is disease more common in any particular month ?	Where does rearer get his seed ?
1	Jote	Pebrine and muscardine	Decreasing . . .	....	August and January .	Nursery.
2	Chhota-Mehdipur .	....	Less disease than formerly.	November 1919 failed (P). April 1—2 annas failed.	Rainy season . .	Nursery usually, sometimes village.
3	Goyesbari . . .	....	Out of 10 rearings one may lose a total. In six years one total loss.	November 1919 total loss (P). Usual April and August 1 to 7 annas loss.	April and August .	Nursery and village.
4	Bamangram . .	Flacherie and muscardine	Worms suffer occasionally from disease.	Nursery seed gives two annas loss. Village seed occasionally 16 annas loss.	Ditto . .	Ditto
5	Mehdipur . .	Pebrine . . .	Sometimes losses at present. Previously more frequent.	....	April, May and rainy season.	Usually village.
6	Gongaprasad . . .	Flacheris and muscardine and other diseases.	Worse cocoons now than 6 years before.	Sometimes 16 annas.	Different bunds . .	Nursery and village.
7	Cahaspara . . .	Pebrine and muscardine	Cocoons improving. Occasionally lose some bunds but has always done so.	November 1919 bad (P). 1920 result from village seed good.	No fixed time . .	Usually village, twice recently Nursery.
8	Moshimpur . . .	All four diseases . .	No pebrine within last 5 years. Ever since he remembers some bunds occasionally fail.	Muscardine gives about two annas loss; other diseases less. November 1919 bad (P); only time in 6 years that nursery seed failed.	Pebrine in April. Muscardine may be all 4 bunds or sometimes not got all year.	Nursery and occasionally village.
9	Ditto . . .	Disease . . .	Not increasing . .	Always 1 to 2 annas loss. Nursery seed good but occasionally one to two annas loss. November 1919 total loss (P).	Cannot say . .	Village and some Nursery.
10	Sujapur . .	Ditto . . .	Ever since he knows about 2 bunds out of 10 fail.	....	April-May and August-September.	Usually Nursery but also village.
11	Mehdipur . . .	Muscardine and grasserie	On previous occasions also some time my silkworms suffered.	About 4 months ago 2 consecutive bunds failed. Is this the November bund ?	Rainy season . .	Nursery always.

APPENDIX II, B.

Nursery seed.

Rearer	Name of village	What diseases are found ?	Are diseases getting more common ? If so, what diseases ?	How much loss is caused by different diseases ?	Is disease more common in any particular month ?	Where does rearer get his seed ?
1	Bilkandi	Rasa, kalshira, chhit, Salfa.	Rasa	10%	August and November	Kalitha Central Nursery.
2	Toilpara	Rasa	Do.	5%	April	Ditto
3	Dhamri	Chhit	Chhit	25%	April, August and November.	Ditto
4	Rameswarpur	Chhit, rasa	Do.	10% to 50%	August and November	Ditto
5	Rampur	Rasa, mathakata, chhit, kalshira.	Rasa	25%	Ditto	Ditto
6	Bachra	Muscardine, flacherie and grasserie.	Yes, muscardine	Almost half of the crop sometimes fails from muscardine and grasserie.	Yes, in the Maghi bund muscardine is almost likely to appear. Flacherie generally appears in the Asari bund.	From Chandpur Central Nursery.
7	Dupukhuria	Grasserie and muscardine	No	Almost one-third of the crop fails from muscardine when it appears and one-fourth from grasserie.	Yes, in the Maghi bund worms are liable to be attacked with muscardine and grasserie.	From Nursery.
8	Bidnpara	Ditto	No	From grasserie and muscardine sometimes one-half of the crop is spoilt.	Yes, in the Sravoni crop grasserie generally breaks out. Muscardine may also appear in the Maghi bund.	Ditto
9	Sompara	Flacherie and grasserie	No	One-third of the crop sometimes fails from flacherie and grasserie.	Yes, in the Asari and Sravoni both grasserie and muscardine may appear.	Ditto
10	Gouripur	Grasserie and muscardine	No	In the rainy season, specially in the Sravoni crop, sometimes one-third of the worms is attacked with the grasserie.	Yes, in the Sravoni crop grasserie may sometimes appear.	Ditto

11	Kumarpur . .	Flacherie or kalshira, lali, hansa or gattine, muscardine or chhit, grasserie or rasa.	Muscardine and grasserie are getting common.	Muscardine causes greatest harm and less harm is caused by grasserie.	(1) Muscardine appears more in the rains (July and August) and in winter, *viz.*, January and February. (2) Flacherie appears more in summer.	Kumarpur Central Nursery.
12	Mahuampur . .	Muscardine, grasserie, flacherie and hansa.	Ditto . .	Ditto . .	(1) Muscardine appears in the rains. (2) Hansa or Salpha or gattine appears in the summer (April and May).	Ditto
13	Chhoto Satui . .	Grasserie, flacherie, kata or pebrine, lali, muscardine, hansa.	Muscardine is getting common.	Ditto . .	(1) Muscardine appears more in the rainy and winter seasons and less in the summer. (2) Grasserie appears more in winter and summer season.	Ditto
14	Kamnagar . . .	Muscardine, flacherie, lali, kata or pebrine, grasserie.	Ditto . . .	Muscardine does greatest harm and less harm is caused by kurkutte or lali.	Outbreak of muscardine is the greatest in the rainy season, *viz.*, July and August.	Ditto
15	Dangapara . . .	Muscardine and grasserie	Grasserie is getting more common.	Grasserie does more harm than muscardine.	Both muscardine and grasserie appear much in the rainy season.	Ditto
16	Shingar-chak . .	Grasserie and muscardine	20 or 25 years ago muscardine was in much prevalence but now gradually disappearing.	One-fourth of the quantity reared.	Grasserie and muscardine are common in May-August and November respectively.	From the Mirganj Central Nursery, District Rajshahi.
17	Manoharpur . .	Grasserie and muscardine were found before the nursery was established. Now scarcely any diseases are found.	Muscardine is gradually disappearing but grasserie is constant.	If muscardine appears in the November-December crops the entire quantity is lost.	Grasserie is common in May and muscardine in November.	Ditto
18	Habashpur . . .	Grasserie and muscardine.	Both grasserie and muscardine are gradually disappearing.	One-eighth of the quantity reared.	Grasserie is common in May and August and muscardine in October and November.	Ditto
19	Barbaria . . .	Muscardine and salpha (gattine).	Muscardine scarcely breaks out but grasserie is common.	About one-fourth or one-sixth of the outturn of the crops raised.	Grasserie in the summer and rainy seasons and muscardine in the winter season.	Ditto

Nursery seed—concld.

Rearer	Name of village	What diseases are found ?	Are diseases getting more common ? If so, what diseases ?	How much loss is caused by different diseases ?	Is disease more common In any particular month ?	Where does rearer get his seed ?
20	Gangarampur . .	Grasserie and salpha (gattine).	Muscardine breaks out almost in every alternate year but grasserie is more or less common.	One-fourth of the quantity reared.	Grasserie in the May and August crops, and muscardine in the November crop.	Mirganj Central Nursery.
21	Atgama . .	Muscardine and grasserie	Muscardine appears frequently.	Loses one-eighth of the crop by these diseases.	Muscardine during Choitra and Baishak; flacherie, grasserie and muscardine in Sravan and Bhadra.	Chandanpur and Kumarpur Central Nurseries.
22	Mohanpara . . .	Flacherie, grasserie and muscardine.	Muscardine and grasserie appear frequently.	Ditto . .	Flacherie and grasserie in Chait and Baishak and muscardine in Bhadra.	Chandanpur, Kumarpur and Piasbari Central Nurseries.
23	Sattari	Flacherie and grasserie .	Grasserie appears frequently.	Loses one-sixteenth of the crop by these diseases.	Flacherie in Chait and Baishak and grasserie in Sravan and Bhadra.	Chandanpur, Kumarpur and Amriti Central Nurseries.
24	Serpur, Dhani . .	Flacherie, grasserie and muscardine.	No disease is getting common.	Loses occasionally one-sixteenth of the crop by these diseases.	Flacherie during Chait and Baishak and grasserie and muscardine during Sravan and Bhadra.	Amriti Central Nursery in general, sometimes from other nurseries.
25	Nabinuggar, Kaliachak	Muscardine and grasserie	Ditto	Loses one-eighth of the crop sometimes by these diseases.	Muscardine during Chait and Baishak and grasserie in Sravan and Bhadra.	Amriti Central Nursery in general. Sometimes Kalitha and Piasbari Central Nurseries.
26	Mehdipore, Malda .	Grasserie, flacherie, muscardine and gattine.	No	Grasserie . 1% Flacherie . . 1% Muscardine . . 5% Gattine . 0·1%	August . Grasserie May . . Gattine	Piasbari Central Nursery.
27	Piasbari, Malda . .	Grasserie, flacherie and muscardine.	No . .	Grasserie=$\frac{1}{10}$. Flacherie—Losses from this disease can be overlooked; muscardine=$\frac{1}{32}$	June . . Grasserie November . Muscardine.	Ditto

28	Kadamtala, Malda .	Ditto . .	No		Grasserie $\frac{1}{10}$, Muscardine $\frac{1}{10}$, Flacherie $\frac{1}{10}$ . .	May . . November . . June . .	Flacherie . . Muscardine. Grasserie	Ditto		
29	Sujapur, Malda .	Grasserie, flacherie, gattine.	muscardine, court and	Grasserie and muscardine.		Grasserie $\frac{2}{10}$, Muscardine, $\frac{1}{10}$ flacherie, court and gattine $\frac{1}{10}$.	November . . June . . May . .	Muscardine. Grasserie Gattine	Ditto	
30	Chandpur, Malda .	Grasserie, and court.	muscardine	Muscardine	. .	Grasserie $\frac{1}{10}$, Muscardine, $\frac{1}{10}$ Court $\frac{1}{10}$ . . .	June . . November . .	Grasserie . . Muscardine.	Ditto	

APPENDIX II, C.

Village seed.

Rearer	Name of village	What diseases are found ?	Are diseases getting more common ? If so, what diseases ?	How much loss is caused by different diseases ?	Is disease more common in any particular month ?	Where does rearer get his seed ?
1	Habashpore . . .	Grasserie, saipha (gattine), and muscardine.	Muscardine commonly breaks out in the rainy season.	¼ of the crop reared .	Grasserie is more common in May and muscardine in August.	Only Chhotapolu seeds are secured from Birbhum District. Nistari seeds are secured from the Mirganj Central Nursery.
2	Kamaldlar . . .	Grasserie and muscardine.	Only muscardine is getting common.	In some crops about ¼ of the quantity reared is lost.	Grasserie and muscardine are very common from June to November	Chhotapolu seeds are secured from Birbhum and Baragharia and Maharajpur in the Murshidabad District.
3	Kadampur . . .	Grasserie and muscardine in a little extent.	Muscardine is very common in November.	In every crop about ⅜ of the crop is lost.	Grasserie and muscardine are common from May to November.	Nistari seeds from Nursery. Chhotapolu seeds from the Birbhum and Murshidabad Districts.
4	Pakuria . . .	Muscardine, grasserie, and flacherie.	Muscardine is common in November.	In every crop about ⅛ is lost.	Grasserie is common from May to July and muscardine is common in November.	Nistari seed from Mirganj Nursery. Chhotapolu seeds from Birbhum and Murshidabad Districts.
5	Balihar . . .	Grasserie and muscardine.	Muscardine is more common than grasserie.	In every crop about ⅛ is lost by grasserie only.	Grasserie is common in June and July. Muscardine in November and December.	Chhotapolu seeds from Murshidabad. Nistari seeds from Mirganj Nursery.
6	Atgram . . .	Rasa, chunakete, kalshira, mathakata, katasa, nona-laga.	Chunakete, mathakata	Wholesale . . .	April . . .	Saktipur (Murshidabad), Mehdinagar (Birbhum).
7	Barla	Nona-laga, rasa, chunakete, kalshira, salpha.	Chunakete . . .	Half of the crop . .	April, August, and November.	Jangipur and Saktipur (Murshidabad).
8	Ailtala . . .	Chhapa-laga, mathakata, rasa, kalshira, salpha.	Chhit and kata . .	⅛ to ¼ of the crop . .	August and November .	Ditto
9	Toilpara . . .	Kalshira, mathakata, gete-rasa.	Mathakata . . .	¼ of the crop . .	Ditto . .	Saktipur (Murshidabad).
	Dhamri . .	Mathakata, chhit, salpha	Chhit and mathakata .	Wholesale . . .	March, August and November.	Ditto

11	Bachra .	Muscardine, flacherie, lali or rangi and grasserie.	Yes, muscardine, lali or rangi and flacherie.	Sometimes ½ of the crop faile from muscardine.	Yes, in the Maghi or November bund, both muscardine and grasserie appear.	From village.
12	Sompara . .	Flacherie, kata or pebrine, salpha and muscardine.	Yes, flacherie, muscardine and kata or pebrine.	Sometimes the entire crop fails from muscardine and kata or pebrine.	Yes, in the Sravonl muscardine appear.	Ditto
13	Dupukhuria . .	Flacherie, grasserie, lali or rangi and salpha.	Yes, grasserie and flacherie.	Sometimes ½ of the crop faile from flacherie.	Flacherie generally appears in the Asari bund.	Ditto
14	Gordowara . .	Kata or pebrine, grasserie and muscardine.	Yes, kata or pebrine and muscardine.	Sometimes the entire crop fails from kata or pebrine and one-half from muscardine.	Muscardine generally appears in the Maghi bund.	Ditto
15	Gouripur . . .	Muscardine and pebrine.	Yes, muscardine and pebrine.	½ of the crop fails from muscardine and sometimes the entire crop fails from pebrine.	Muscardine appears in Sravoni and Maghi crop.	Ditto
16	Maula . . .	Muscardine, lali, grasserle, kata or pebrine.	Muscardine is getting common.	Lali causes greatest harm; then muscardine and grasserie cause less harm.	Muscardine prevails much in the rainy season, *viz.*, July and August.	Saktipur, Gauripur, Dupkur of the Murshidabad District.
17	Jhumka . . .	Muscardine or chhit, lali or rangi, rasa or grasserie.	Muscardine is getting more common, then comes grasserie.	Muscardine causes greatest harm.	Muscardine much appears in the rainy and winter seasons.	Narkulbari, Dupkur, Bachra, Gauripur, etc., in the district of Murshidabad.
18	Nagar	Muscardine, hansa or gattine or salpha, grasserie, lali.	Muscardine is getting more common.	Ditto . . .	Muscardine appears more in the rainy season and hansa or gattine appears more in the summer season.	Ditto
19	Debkundu . . .	Lali, flacherie, chunakette, or muscardine, grasserie, pebrine or kata, hansa.	Chunakette or muscardine is getting common.	Chunakette or muscardine does greatest harm and flacherie comes next.	Ditto . .	Ditto
20	Mirzapur . . .	Muscardine (whitish and reddish, latter is more harmful), lali, hansa, flacherie, grasserie.	Muscardine of reddish variety is becoming common.	Muscardine does greatest harm and then comes grasserie.	Ditto . . .	Ditto
21	Jote	Pebrine, grasserie, flacherie and muscardine.	Pebrine and muscardine.	Grasserie $\frac{8}{15}$. Pebrine . $\frac{4}{15}$. Muscardine $\frac{1}{15}$. Flacherie. $\frac{2}{15}$.	Flacherie and grasserie from Baishakh to Bhadra, pebrine and muscardine from Agrahayan to Chaitra.	From different villages of the districts of Rajshahi, Murshidabad, Birbhum and Malda.

Village seed—concld.

Rearer	Name of village	What diseases are found ?	Are diseases getting more common ? If so, what diseases ?	How much loss is caused by different diseases ?	Is disease more common in any particular month ?	Where does rearer get his seed ?
22	Nawadabazar . .	Pebrine, grasserie, flacherie and muscardine.	Pebrine and grasserie common, flacherie occasional.	Sometimes whole crop fails and sometimes about half of the crop fails by different diseases.	Pebrine, grasserie and flacherie during Chaitra to Bhadra, pebrine and muscardine during Agrahayan to Chaitra.	From different villages of the districts of Birbhum, Rajshahi and Malda.
23	Garkhola . . .	Pebrine, grasserie and muscardine.	Pebrine and grasserie.	Sometimes whole crop fails by pebrine and sometimes about one-fourth of the crop fails by muscardine.	Grasserie and muscardine in Baishakh to Bhadra, pebrine and muscardine in Agrahayan to Chaitra.	From the different villages of Murshidabad and Birbhum Districts.
	Chhabilpara . .	Pebrine, grasserie and flacherie.	Pebrine and grasserie, flacherie occasional.	Sometimes whole crop fails by pebrine and sometimes $\frac{1}{4}$ of the crop fails by grasserie and other diseases.	Grasserie and flacherie in Baishakh to Bhadra, pebrine and grasserie from Agrahayan to Chaitra.	From the different villages of the districts of Rajshahi, Birbhum and Murshidabad.
25	Dullugram . . .	Pebrine, grasserie and flacherie.	Pebrine common. Grasserie and flacherie occasional.	Sometimes whole crop fails by pebrine and sometimes $\frac{1}{4}$ of the crop fails by pebrine, flacherie and grasserie.	Grasserie, flacherie and pebrine in Baishakh to Bhadra and pebrine and grasserie in Agrahayan to Chaitra.	From the different villages of the districts of Birbhum; Murshidabad and Malda. Having been totally discouraged by village seeds, he has taken seeds for the first time for his current Bhaduri crop from the Amriti Central Nursery.
26	Mehdipore, Malda . .	Muscardine, grasserie, pebrine, pebrine with grasserie, gattine, flacherie.	Grasserie with pebrine.	Grasserie with pebrine=$\frac{2}{16}$ Whenever severe type of muscardine breaks the worms are thrown away during their younger stages. Flacherie=$\frac{1}{8}$ Losses from other diseases can be overlooked.	November — Muscardine and pebrine. April—Muscardine and gattine. May—Grasserie and flacherie. August—No disease. September—Pebrine and grasserie with pebrine.	Nistari seed—Birbhum, Murshidabad and Malda. Chhotapolu seed—Murshidabad.
	Harirampore, Malda .	Grasserie, muscardine, flacherie, court.	Grasserie and court.	Grasserie=$\frac{1}{4}$ Muscardine=$\frac{1}{4}$ Losses from other diseases can be overlooked.	November—Muscardine. June—Grasserie.	Nistari eeed—Maharajganj. Joar, Malda and Kausat and Shibganj side, Malda. Chhotapolu—Birbhum.

28	Sultanpore, Malda .	Grasserie, muscardine, court, gattine.	Muscardine appears in almost every crop.	Grasserie=$\frac{1}{16}$ Muscardine=$\frac{1}{16}$ Losses from other diseases are negligible.	June—Grasserie November—Muscardine.	Nistari seed—Chandpore and Haripore chatra, Malda. Chhotapolu seed—Rarh (Birbhum).
29	Shasni, Malda . .	Grasserie, flacherie .	During extremely hot weather flacherie appears.	Flacherie=$\frac{1}{16}$	May and August—Flacherie.	Nistari seed—Maharajpore, Joar, Malda. Chhotapolu seed—Rarh (Birbhum).
30	Naya tola, Malda . .	Grasserie, muscardine .	Grasserie in June	Grasserie=$\frac{1}{16}$ Muscardine=$\frac{1}{16}$	Grasserie during June and July crop.	Ditto

APPENDIX III.

EXPERIMENT 1.

Amount of disease in progeny of diseased moth. 1st generation of diseased moth's progeny.

Lot	Race and origin of seed	Rearing period	Per cent. pebrine	Weight of 10 empty cocoons	REMARKS
				grm.	
1	Hybrid-parent moth infected in caterpillar stage.	9-9-20 to 1-10-20	12·5 (16)	1·115	In these and all succeeding experiments the numbers in brackets are the numbers of moths examined.
2	Ditto ditto .	9-9-20 to 29-9-20	10 (30)	0·950	
3	Nistari-parent moth infected in caterpillar stage.	12-11-20 to 13-1-21	37 (27)	0·394	

Experiment 2.

Amount of disease in progeny of diseased moth. 2nd generation of diseased moth's progeny.

Lot	Race and origin of seed	Rearing period	Per cent. pebrine	Weight of 10 empty cocoons	Remarks
				grm.	
1	Nistari-parent moth heavily diseased and itself the offspring of a diseased moth (31).	18-6-20 to 7-7-20	61·2	0·850	
2	Ditto ditto (26) .	19-6-20 to 10-7-20	69·2	0·705	
3	Hybrid race-parent moth heavily diseased and itself the offspring of a diseased moth (50).	8-9-20 to 28-9-20	16	1·090	
4	Nistari-parent moth heavily diseased and itself the offspring of a diseased moth (47).	11-9-20 to 3-10-20	23·4	0·927	
5	Ditto ditto (42) .	13-11-20 to 12-1-21	50	.....	

Experiment 3.

Amount of disease in progeny of diseased moth. 3rd generation of diseased moth's progeny.

Lot	Race and origin of seed	Rearing period	Per cent. pebrine	Weight of 10 empty cocoons	Remarks
				grm.	
1	Nistari-parent moth heavily diseased and the two previous generations from diseased moths (132).	26-7-20 to 17-8-20	96·2	0·750	The previous generation was Lot 2, Experiment 2. There were 63 dead pupæ in the lot—all pebrinised.
2	Ditto ditto (73) .	20-10-20 to 15-11-20	49·3	1·035	The previous generation was Lot 4, Experiment 2. 11 dead pupæ were got—10 pebrinised.
3	Ditto ditto (27) .	20-10-20 to 10-11-20	77·7	0·640	20 dead pupæ were got—all pebrinised.

Experiment 4.

Amount of infection in highly infected house.

Lot	Race and origin of seed	Rearing period	House infected	Worms put in	Per cent. pebrine	Control	Remarks
1 (32)	Nistari . .	11-5-20 to 3/4-6-20	14-5-20	15-5-20 1st moult	12·5	36—No pebrine	Material used for infecting house when sprinkled directly on worms gave 15·7% pebrine.
2 (47)	Do. . .	19-6-20 to 7/8-7-20	18-6-20	19-6-20 On hatching	10·6	37—No pebrine	Material used for infecting house when sprinkled directly on worms gave 10·7% pebrine.
3 (23)	Do. . .	22-7-20 to 9/10-8-20	19-7-20	22-7-20 On hatching	12·5	25—No pebrine	Material used for infecting house when sprinkled directly on worms gave 48·5% pebrine.
4 (67)	Hybrid (Berhampore)	16-7-20 to 3-8-20	19-7-20	20-7-20 After 1st moult	17·3	50—No pebrine	Fed direct as smear on 3 occasions gave 100% pebrine.
5 (70)	Nistari . .	29-8-20 to 17/18-9-20	30-8-20	1-9-20 After 1st moult	40	25—No pebrine	
6 (48)	Do. . .	5-10-20 to 23/24-10-20	3-10-20	5-10-20 On hatching	6·2	25—No pebrine	

Experiment 5.

Amount of infection in infected tray.

Lot	Race and origin of seed	Date of hatching	Dates of moults	Date of spinning	Tray infected	Worms put in tray	Per cent. pebrine	Wt. 10 empty cocoons	Control
1	Nistari (95) .	20-6-20	24-6-20 26-6-20 29-6-20 3-7-20	8/9-7-20	21-6-20	22-6-20 2 days after hatching.	15·8	grm. 1·070	42 disease-free.
2	Do. (23) .	20-6-20	24-6-20 26-6-20 29-6-20 3-7-20	8/9-7-20	22-6-20	26-6-20 After 2nd moult.	34·7	0·995	42 disease-free.
3	Hybrid Nistari × Mysore (12)	3-8-20	6-8-20 9-8-20 12-8-20 16-8-20	22/23-8-20	6-8-20	11-8-20 Just before 3rd moult.	83·3	1·065	55 disease-free.
4	Nistari (12) .	29-8-20	1-9-20 4-9-20 7-9-20 11-9-20	17/18-9-20	31-8-20	2-9-20 Just after 1st moult.	58·3	1·080	25 disease-free.
5	Do. (23) .	26-8-20	29-8-20 1-9-20 4-9-20 8-9-20	13/14-9-20	27-8-20	1-9-20 Just after 2nd moult.	100	1·035	35 disease-free.
6	Do. (12) .	12-11-20	18-11-20 23-11-20 29-11-20 1-12-20	20/21-12-20	9-11-20	18-11-20 After hatching.	78·5	...	40 disease-free.
7	Do. (12) .	5-10-20	8-10-20 11-10-20 14-10-20 17-10-20	23/24-10-20	8-10-20	11-10-20	...	1·200	

Experiment 6.

Amount of infection in infected mulberry plantation.

Lot	Race and origin of seed	Date of hatching	Date of moults	Date of spinning	Plantation infected	Leaf fed to worms.	Per cent. pebrine	Wt., 10 empty cocoons
1	Nistari × Mysore Hybrid (124)	3-8-20	6-8-20 9-8-20 12-8-20 16-8-20	23/24-8-20	12-7-20 and 27-7-20	From hatching	87	grm. 0·920
1a	Ditto (55)	Do.	Do.	22/23-8-20	Normal leaf	...	No pebrine	1·125
2	Nistari × Mysore Hybrid (35)	8-9-20	11-9-20 14-9-20 17-9-20 21-9-20	27-9-20	6-9-20	9-9-20	11·4	1·150
2a	Ditto (29)	Do.	Do.	26/27-9-20	Normal leaf	...	No pebrine	1·300
3	Nistari × Mysore Hybrid (40)	14-10-20	17-10-20 20-10-20 24-10-20 28-10-20	4/5-11-20	3-10-20 Manure once previous to this but date not noted.	20-10-20	85	1·260
3a	Ditto (76)	Do.	Do.	3-11-20	Normal leaf	...	No pebrine	1·370

Experiment 7.

Amount of infection acquired from diseased worms.

Lot	Race and origin of seed and No. of healthy worms used	Rearing period	No. of diseased worms	How diseased worms were infected	Diseased worms infected	Worms put together	No. of moths found infected (pebrinised)	Control
1	Nistari (18)	26-8-20 to 16-9-20	6	By smearing mouths with fresh peb. material.	1-9-20 and 4-9-20	4-9-20 to 16-9-20	24—All worms became infected.	Disease-free (42).
2	Do. (12)	9-11-20 to 19-12-20	3	By smearing mouths with fresh peb. material.	26-11-20	27-11-20 to 19-12-20	5—Only 2 worms became infected.	Disease-free (58).
3	Hybrid race (40)	8-9-20 to 27-9-20	40	2nd generation of diseased moth's progeny, mother moth highly diseased. Diseased control gave 16% pebrine (50).	...	8-9-20 to 27-9-20	21 out of 67 giving 31·3% pebrine. 13 worms were lost during 1st stage.	Disease-free (43).
4	Do. (30)	9-9-20 to 29-9-20	30	1st generation of diseased moth's progeny, mother moth highly diseased. Diseased control gave 10% pebrine.	...	9-9-20 to 29-9-20	6 out of 53 giving 11·3% pebrine. 7 worms were lost during 1st stage.	Disease-free (30).

Experiment 8.

Length of time pebrinised material remains infective.

Lot	Date of finding material infective	Date of feeding	Length of time	How fed	Rearing period	Per cent. pebrine	Control
1 (16)	26-3-20	26/27-5-20	8 weeks 5 days	Leaf smear 2 meals	14-5-20 to 7/8-6-20	68·7	14—No peb.
2 (12)	Do.	26/27-6-20	13 weeks 1 day	Leaf smear 3 meals	16-6-20 to 6-7-20	nil	49—No peb.
3 (37)	Do.	27/28-6-20	13 weeks 2 days	Ditto	21-6-20 to 8/9-7-20	nil	49—No peb.

Experiment 9.

Length of time infected house remains infective.

Lot	Race	Date of infecting house	Date of putting in worms	Length of time since infection	Rearing period	Per cent. pebrine	Control
1 (32)	Nistari . .	18-6-20	19-6-20	1 day	19-6-20 to 3/4-7-20	12·5	36—No peb.
1a (51)	Do. . .	Do.	22-7-20	4 weeks 6 days . .	22-7-20 to 9/10-8-20	1·9	25—No peb.
2 (47)	Do. . .	19-7-20	22-7-20	3 days. . . .	22-7-20 to 9/10-8-20	12·5	25—No peb.
2a (39)	Do. . .	Do.	1-9-20	6 weeks 2 days . .	29-8-20 to 17/18-9-20	12·8	25—No peb.
2b (51)	Do. . .	Do.	1-10-20	10 weeks 4 days . .	1-10-20 to 19/20-10-20	3·9	25—No peb.
2c (14)	Do. . .	Do.	10-11-20	16 weeks 2 days . .	10-11-20 to 18/20-12-20	nil	101—No peb.
3 (70)	Do. . .	30-8-20	1-9-20	2 days . . .	29-8-20 to 17/18-9-20	40	25—No peb.
3a (34)	Do. . .	Do.	1-10-20	4 weeks 4 days . .	23-9-20 to 19/20-10-20	5·8	25—No peb.
3b (77)	Do. . .	Do.	10-11-20	10 weeks 2 days . .	10-11-20 to 17/18-12-20	nil	101—No peb.
4 (48)	Do. . .	3-10-20	5-10-20	2 days . . .	5-10-20 to 23/24-10-20	6·2	25—No peb.
4a (44)	Do. . .	Do.	10-11-20	5 weeks 3 days . .	10-11-20 to 18/20-12-20	nil	101—No peb.

Experiment 10.

Effect of shielding worms in infected house under a paper box.

Lot	Race	Date of infecting house	Date of putting in worms	Rearing period	Per cent. pebrine under box	Per cent. pebrine in open tray	Control
1	Nistari . .	14-5-20	15-5-20	11-5-20 to 1/2-6-20	(34) nil	12·5 (32)	Disease-free (24).
2	Do. . .	18-6-20	9-6-20	19-6-20 to 7-7-20	(51) nil	10·6 (47)	Disease-free (37).

Experiment 11.

To test effect of sun's rays on powdered pebrinised material.

Lot	Race and origin of seed	Method of treating material	How fed	Rearing period	Per cent. pebrine	Normal control
1 (17)	Nistari . .	Untreated	3 meals on leaf after 3rd moult	14-5-20 to 7/8-6-20	17·5	(14) Disease-free.
2 (18)	Do. . .	Exposed to direct sun's rays on three days—18 hours in all.	Ditto	Ditto	nil	Do.
3 (14)	Do. . .	Exposed to sun in enclosed wooden box for 18 hours on 3 days.	Ditto	Ditto	nil	Do.

Experiment 12.

Effect of sun's rays on infected trays.

Method of sterilization	Date of putting worms in trays	Rearing period	Per cent. pebrine	Per cent. pebrine in untreated tray	Normal control
Exposed to direct sun's rays for 18 hours (3 days).	26-6-20 After 2nd moult.	20-6-20 to 8/9-7-20	25·6 (35)	34·7 (23)	(42) No peb.
Exposed to direct sun's rays for 24 hours (4 days)	22-6-20 Before 1st moult.	20-6-20 to 8/9-7-20	nil (80)	15·8 (95)	Ditto
Exposed to direct sun's rays for 36 hours (4 days).	11-8-20 Before 3rd moult.	3-8-20 to 22/23-8-20	66·6 (12)	83·3 (12)	(55) No peb. Nistari × Mysore worms were under the experiment.
Exposed to direct sun's rays for 34 hours (4 days).	1-9-20 At 2nd moult.	26-8-20 to 13/14-9-20	25 (24)	100 (23)	(35) No peb.
Exposed to direct sun's rays for about 18 hours (4 days).	12-11-20 At hatching.	12-11-20 to 20/21-12-20	11·7 (17)	78·5 (14)	(40) No peb.

Experiment 13.

Effect of various germicides on infected trays.

Method of sterilization	Date of putting worms in trays	Rearing period	Per cent. pebrine	Per cent. pebrine in untreated tray	Normal control
Smearing with cowdung . . .	2-9-20 After 1st moult.	29-8-20 to 17/18-9-20	58·3 (12)	58·3 (12)	(25) No peb.
2% Copper sulphate spray . . .	12-11-20 On hatching.	12-11-20 to 20/21-12-20	33·3 (13)	78·5 (12)	(40) No peb.
2% Copper sulphate spray and sulphur fumes for 1 hour.	Ditto	Ditto	80 (8)	Ditto	Ditto
2% Copper sulphate and sun's rays for 1 day.	Ditto	Ditto	50 (14)	Ditto	Ditto
1% Formalin spray	Ditto	Ditto	42·8 (14)	Ditto	Ditto
1% Formalin spray and sun for 1 day	Ditto	Ditto	9 (33)	Ditto	Ditto

Experiment 14.

To test effect of germicides directly on powdered pebrinised material. Material treated about a day before feeding.

Germicide	How used	Date of feeding material	Rearing period	Per cent. pebrine	Per cent. pebrine in untreated control	Control	Remarks
1% Formalin	Heavily sprayed	3/5-9-20 in loopfuls	26-8-20 to 14/15-9-20	16·6 (12 moths)	100 (12 moths)	42 caterpillars disease-free.	10 empty cocoons, weight 1·150 grm.
1% Formalin	Normally sprayed	3/5-9-20 in loopfuls	26-8-20 to 14-9-20	100 (12 moths)	100	Do.	10 empty cocoons, weight 1·060 grm.
1 % Copper sulphate.	Sprayed	3/5-9-20 in loopfuls	26-8-20 to 15/16-9-20	100 (12 moths)	100	Do.	10 empty cocoons, weight 0·920 grm.

The above experiment was repeated in the following month and gave similar results but as several of the caterpillars got lost during the experiment, the details are not given.

The experiment was repeated 3 times in Kalimpong but in every case the untreated material gave such a low percentage of infection that the results were of little value and are not given—the sprayed material gave no disease, while the unsprayed gave either none or at most 16·6% pebrine.

Experiment 15.

Testing various germicides in infected houses.

Lot	Race	Germicide	Date of infecting house	Date of disinfecting	Worms put in house	Rearing period	Per cent. pebrine	Per cent. pebrine in undisinfected house	Control
1	Nistari .	1% Formalin spray	14-5-20	14-5-20	15-5-20	11-5-20 to 2/3-6-20	1·8 (53)	12·5 (32)	Disease-free (36).
2	Do. .	1% Copper sulphate spray.	18-6-20	19-6-20	19-6-20	19-6-20 to 15/16-7-20	4·8 (41)	10·6 (47)	Disease-free (37).
	Do. .	Wood smoke and formaldehyde fumes	18-6-20	19-6-20	19-6-20	19-6-20 to 15/16-7-20	nil (40)	10·6 (47)	Do.
4	Do. .	Sulphur fumes	19-7-20	20-7-20	22-7-20	22-7-20 to 8/9-8-20	10·7 (28)	12·5 (23)	Disease-free (25).
5	Do. .	1% Formalin spray	30-8-20	31-8-20	1-9-20	29-8-20 to 17/18-9-20	2·8 (35)	40 (70)	Do.

EXPERIMENT 16.

Effect of moisture on pebrine.

Lot	Race and origin of seed	Atmesphere	Treatment	Temperature range during day	Humidity range during day	Hatching date	Dates of moults	Spinning date	Per cent. disease	REMARKS
1 (17)	Nistari . .	Dry .	Normal	75°-95° F.	19%—82%	2-4-20	6-4-20 11-4-20 15-4-20 20-4-20	26/27-4-20	nil	1 and 2 were from the same laying.
2(40)	Do. . .	Moist .	Do.	74°-93° F.	56%—97%	2-4-20	6-4-20 10-4-20 14-4-20 18-4-20	24-4-20	41·2 Fl.	
3 (25)	Do. .	Dry .	2 infected meals after 3rd moult.	75°-95° F.	19%—82%	2-4-20	6-4-20 11-4-20 16-4-20 21-4-20	28/29-4-20	24 Peb.	3 and 4 were from the same laying.
4 (31)	Do. . .	Moist .	Do.	74°-93° F.	56%—97%	2-4-20	6-4-20 10-4-20 14-4-20 18-4-20	24/25-4-20	83·8 Peb. 35·4 Fl.	
5 (64)	Do. . .	Dry .	Infected cage	75°-95° F.	19%—82%	2-4-20	6-4-20 11-4-20 15-4-20 20-4-20	27/28-4-20	1·5 Peb.	5 and 6 were from the same laying.
6 (45)	Do. . .	Moist .	Do.	74°-93° F.	56%—97%	2-4-20	6-4-20 10-4-20 14-4-20 18-4-20	24-4-20	22·2 Peb. 46·6 Fl.	

Experiment 17.

Effect of moisture on pebrine.

Lot	Race and origin of seed	Atmosphere	Treatment	Temperature range during day	Humidity range during day	Hatching date	Date of moults	Spinning date	Per cent. disease	Remarks
1 (54)	Nistari . .	Dry .	Normal	81°-93° F.	56%—91%	11-5-20	15-5-20 18-5-20 23-5-20 27-5-20	3/4-6-20	nil	1 and 2 were from the same laying.
2 (17)	Do. . .	Moist .	Do.	82°-95° F.	72%—100%	11-5-20	15-5-20 18-5-20 21-5-20 25-5-20	30/31-5-20	nil	
3 (48)	Do. . .	Dry .	2 infected meals after 2nd moult.	81°-93° F.	56%—91%	11-5-20	15-5-20 18-5-20 23-5-20 28-5-20	3/5-6-20	14·5 Peb.	3 and 4 were from the same laying.
4 (42)	Do. . .	Moist .	Do.	82°-95° F.	72%—100%	11-5-20	15-5-20 18-5-20 24-5-20 28-5-20	3/4-6-20	90·4 Peb.	
5 (35)	Do. . .	Dry .	Infected cag	81°-93° F.	56%—91%	11-5-20	15-5-20 18-5-20 23-5-20 28-5-20	3/4-6-20	nil	5 and 6 were from the same laying.
6 (58)	Do. . .	Moist .	Do.	82°-93° F.	72%—100%	11-5-20	15-5-20 18-5-20 21-5-20 25-5-20	31-5-20 to 1-6-20	nil	

Experiment 18.

Effect of number of meals on resistance to pebrine.

Lot	Race and origin of seed	How reared	No. of meals	Hatching date	Dates of moults	Spinning date	Per cent. pebrine	Remarks
1a (17)	Nistari . .	Normal	6	2-4-20	6-4-20 11-4-20 15-4-20 20-4-20	26/27-4-20	nil	
1b (60)	Do. . .	Infected cage	Fresh food always in front of worms.	2-4-20	6-4-20 10-4-20 15-4-20 20-4-20	24/27-4-20	nil	
1c (32)	Do. . .	Ditto	4	2-4-20	6-4-20 11-4-20 16-4-20 20-4-20	28/29-4-20	12·5	
2a (17)	Do. . .	Normal	6	14-5-20	18-5-20 23-5-20 27-5-20 31-5-20	7/8-6-20	nil	
2b (23)	Do. . .	1 peb. meal given on leaf—19-5-20.	Fresh food always in front of worms.	14-5-20	18-5-20 23-5-20 27-5-20 31-5-20	7/8-6-20	nil	
2c (13)	Do. .	Ditto	4	14-5-20	18-5-20 24-5-20 28-5-20 2-6-20	9/11-6-20	nil	Somewhat irregular and slower.

Experiment 19.

Effect of food on diseased moths' progeny.

Lot	Race and origin of seed	Food given	Rearing period	Caterpillar mortality per cent.	Per cent. of cocoons got	Per cent. of moths got	Per cent. pebrine	Weight of 10 empty cocoons	Remarks
								grm.	
1a (19)	Nistari—one laying of 1st gen. diseased moths' progeny.	4 meals, bush	12-11-20 to 28-12-20/19-1-21	34·7	65·2	17·7	26·6	0·370	See Plate IV. Note high caterpillar mortality and small number of moths got.
1b (34)	Ditto	6 meals, bush	12-11-20 to 26-12-20/13-1-21	20·5	79·4	51·8	37	0·394	See Plate V.
1c (68)	Ditto	6 meals, tree	12-11-20 to 25-12-20/16-1-21	4·40	94·2	69·2	35·3	0·433	See Plate VI. Note low caterpillar mortality and large number of cocoons and moths got.
2a (31)	Nistari—one laying of 1st gen. diseased moths' progeny.	4 meals entire twigs	9-9-20 to 1/5-10-20	..	100	100	16·1	0·827	Caterpillar weights— grm. Smallest . . 0·077 Intermediate . 0·200 Largest . . 0·579
2b (19)	Ditto	4 meals, picked leaf	9-9-20 to 30-9-20/4-10-20	..	100	84·2	31·5	0·920	Smallest . . 0·168 Intermediate . 0·279 Largest . . 1·065
2c (16)	Ditto	6 meals, picked leaf	9-9-20 to 30-9-20/1-10-20	..	100	100	12·5	1·115	Smallest . . 0·172 Intermediate . 0·801 Largest . . 1·272
2d (23)	Ditto	6 meals daily sprinkled with sterile dust.	9-9-20 to 30-9-20/4-10-20	..	100	100	13	1·105	

Experiment 20.

Effect of food on normal caterpillars.

Lot	Race and origin of seed	Food given	Hatching date	Date of moults	Spinning date	Per cent. disease	Weight of 10 empty cocoons	Remarks
							grm.	
1 (12)	Nistari—Mass selection, Lot 1.	4 meals, bush	21-7-20	25-7-20 28-7-20 31-7-20 4-8-20	9/10-8-20	nil	1·025	Caterpillars rather irregular.
2 (47)	Nistari—Mass selection, Lot 5.	Sterile dust on leaf	22-7-20	25-7-20 28-7-20 31-7-20 3-8-20	9/10-8-20	nil	0·095	Very poor cocoons.
(25)	Ditto	6 meals, bush	21-7-20	25-7-20 28-7-20 31-7-20 3-8-20	8/9-8-20	nil	1·350	

Experiment 21.

To test the value of hill rearing.

Lot	Race	Origin of seed	Where reared	Rearing period	Treatment of worms	Per cent. pebrine	Remarks
1	Nistari (112)	Reared 3 generations in Kalimpong.	Kalimpong . .	10-7-21 to 5-8-21	Reared in infected cage.	89·2	
2	Do. (57)	Ditto . . .	Pusa . . .	7-7-21 to 26-7-21	Ditto . .	78·9	
3	Do. (66)	Pusa seed . . .	Do. . . .	18-7-21 to 6-8-21	Ditto . .	49	
4	Do. (75)	Ditto . . .	Do. . . .	18-7-21 to 5-8-21	Normal rearing .	1·3	The cage containing these worms was reared beside the infected cage. This probably accounts for % of pebrine.
1a	Do. (67)	Reared 4 generations in Kalimpong.	Kalimpong . .	26-8-21 to 18-9-21	Reared in infected cage.	8·9	
2a	Do. (65)	Ditto . . .	Pusa . . .	24-8-21 to 14-9-21	Ditto . .	7·6	
3a	Do. (54)	Pusa seed . . .	Do. . . .	22-8-21 to 12-9-21	Ditto . .	9·2	
4a	Do. (100)	Ditto . . .	Do. . . .	22-8-21 to 9-9-21	Normal rearing .	nil	

Experiment 22.

To see if spores are more numerous in moths five days after cutting out than on day of cutting out.

Lot		Age of moth	No. examined	No. pebrinised	Proportion of spores to meronts
1	Diseased moth's progeny .	Day of cutting out . .	25	23	Not noted.
	1 stained slide was made from each lot and examined for meronts.	1 day after cutting out . .	25	21	Spores more numerous than meronts.
		2 days after cutting out .	25	17	Ditto
		3 „ „ „ „ .	25	20	Many spores—very few meronts.
		4 „ „ „ „ .	5	4	No parasites in stained specimens.
		5 „ „ „ „ .	5	3	Spores only.
		6 „ „ „ „ .	1	1	Many spores—very few meronts.
		7 „ „ „ „ .	1	1	Ditto
		8 „ „ „ „ .	1	1	Spores only.
2	Caterpillars fed heavily on infective material. All specimens were stained and examined for meronts.	Day of cutting out . .	1	1	Many spores—very few meronts.
		1 day after cutting out . .	5	5	Many spores—not so many meronts.
		2 days after cutting out .	4	4	Many spores—very few meronts.
		3 „ „ „ „ .	4	4	Ditto
		4 „ „ „ „ .	3	3	Many spores—not so many meronts.
		5 „ „ „ „ .	4	4	Many spores—meronts equally numerous.

Experiment 23.

To see if flacherie is produced by feeding caterpillars on putrifying material from dead caterpillars.

Lot	Race used	Organism fed	Origin of organism	When fed	Caterpillar mortality	Examination of moths	Control
1	Nistari (6) . .	Chiefly bacillus .	Fed direct from caterpillar that had died of pebrine and flacherie.	26-11-20	.....	2 pebrine; no flacherie.	No disease (25).
2	Ditto . .	Ditto . .	Ditto	12/13-12-20	1 missing . . .	5 pebrine; no flacherie.	Do.
3	Hybrid (1) .	Ditto . .	Fed direct from caterpillar that had died of flacherie.	15-1-21	.....	Moth failed to emerge—no organism found.	No disease.
4	Hybrid (1) (Berhampore).	Ditto . .	Ditto	29-1-21	Died on 4th February 1921, no bacilli—a few diplococci got.	...	Do.
5	Hybrid (4) .	Ditto . .	Ditto	14-1-21	3 died on 20th and 21st January 1921, becoming discoloured and flaccid after death but only one showed bacillus in blood and gut.	1 pupa; no organism found.	Do.
6	Hybrid (6) .	Bacillus and micrococcus.	Ditto	9-1-21	4 died on 10th, 21st and 22nd January 1921, becoming discoloured and flaccid after death; one showed bacilli and cocci.	2 dead pupæ; one showed bacilli and many cocci; the other cocci.	Do.
7	Ditto . .	Mostly bacillus .	Ditto	9-1-21	3 died on 19th, 20th and 21st January 1921, becoming discoloured and flaccid after death. One showed cocci in gut and blood. Other two showed bacilli.	3 moths; one showed cocci.	Do.

Experiment 24.

To see if flacherie is produced by feeding mulberry caterpillars on organisms recovered from diseased Muga caterpillars.

Lot	Race	Organism fed	Origin of organism	When fed	Caterpillar mortality	Examination of moths	Control
1	Nistari (5) . .	Mixed Bacillus A and Micrococcus a.	Agar slope from diseased Muga worms (flacherie).	14-4-21	.....	Flacherie nil, peb. 1.	No disease.
2	Ditto . .	Bacillus A . .	Agar slope of 17th April 1921.	26-4-21	.....	nil	Do.
3	Ditto . .	Ditto . .	Nutrient broth culture of 14th April 1921.	26-4-21	.....	nil	Do.
4	Nistari (2) . .	Mixed Bacillus A and Micrococcus a.	Agar slope of 6th April 1921.	25-4-21	.....	nil	Do.
5	Nistari (5) . .	Micrococcus a .	Agar plate of 19th April 1921.	26-4-21	.....	nil	Do.
6	Nistari (6) . .	Ditto . .	Ditto . .	25-4-21	.....	nil	Do.
7	Chhotapolu (12) .	Bacillus A . .	Agar slope of 12th May 1921.	1-6-21	.....	nil	No disease (50).
8	Ditto . .	Micrococcus a .	Ditto . .	1-6-21	.....	nil	Do.

EXPERIMENT 25.

To see if flacherie is produced by feeding bacteria isolated from various sources to caterpillars under normal conditions.

Lot	Race	Organism fed	Origin of organism	When fed	Caterpillar mortality	Examination of moths	Control	REMARKS
1	Chhotapolu (12)	Bacillus B	From moth with flacherie—agar slope of 12th May 1921.	1-6-21	.....	3 died 4th day after emerging—2 showing Bacillus B. Other 9 alive on examination and showed no bacteria.	No disease (50)	
2	Ditto .	Bacillus A	From mulberry leaf, agar slope of 12th May 1921.	1-6-21	....	2 died on 2nd day showed Bacillus A. Rest alive on examination and showed no organism.	Ditto	
3	Ditto .	Micrococcus a	From mulberry leaf and healthy caterpillar, agar slope of 12th May 1921.	1-6-21	....	None showed any bacteria and were alive when examined.	Ditto	
4	Ditto .	Micrococcus b	Ditto	1-6-21	....	Ditto	Ditto	
5	Ditto .	Micrococcus c	Ditto	1-6-21	....	1 moth failed to emerge. Rest alive on examination ; no bacteria.	Ditto	
6	Nistari (6)	Bacillus B	From moth with flacherie, agar slope of 2nd July 1921.	8-7-21	One died on 22nd July 1921, very flaccid and slightly discoloured but no bacteria. Gut less alkaline than normal (pH=8·6).	4 normal	4 Pebrioc No Fl. (53)	The control was reared on a stand alongside of diseased caterpillars.
7	Ditto . .	Bacillus A	From Muga worm died of flacherie, agar slope of 2nd July 1921.	8-7-21	....	1 Peb. 5 normal		
8	Ditto . .	Micrococcus a	Ditto	8-7-21	....	1 Peb. 5 normal.		
	Ditto . .	Bacillus A	From Kashmir univoltine mulberry worm died of flacherie, agar slope of 2nd July 1921.	8-7-21	....	All normal		

10	Ditto	.	.	Bacillus A	As 9 but grown under anærobic conditions, agar slope of 14th July 1921.	16-7-21	1 died 19th July 1921. Black and rotten Bacillus A in large numbers Gut=pH=8·8.	5 normal	.
11	Ditto			Micrococcus a	From Kashmir univoltine mulberry worm died of flacherie, agar slope of 2nd July 1921.	8-7-21	....	All normal	
12	Ditto	.	.	Bacillus C	Ditto	8-7-21	....	Ditto	

EXPERIMENT 26.

To see if flacherie is produced by feeding bacteria isolated from various sources to caterpillars kept in chambers at 95° F. and saturated with water vapour.

Lot	Race	Organism fed	Origin of organism	When fed	Caterpillar mortality	Control in hot moist chamber	Control under normal conditions
1	Nistari (15) . .	Bacillus A Bacillus C Micrococcus a	Univoltine caterpillars from Kashmir that had died of flacherie (May 1921), agar slope of 29th June 1921.	1-7-21 Just after passing 3rd moult.	9 died 2nd July 1921. All symptoms of flacherie. All organisms fed present in large numbers. Gut very acid (pH=2·4). 6 died on passing 4th moult—a few micrococci present. Gut slightly less alkaline than normal (pH=8·8).	Many died on passing 3rd moult (50). All remaining died on passing 4th moult with symptoms of flacherie (15). Gut somewhat less alkaline than normal (pH=8·4, 8·8). 3 caterpillars showed a few micrococci	No disease (53).
2	Nistari (6) . .	Bacillus A	As lot 1 but agar slope of 2nd July 1921.	6-7-21	2 died on 9th July 1921; one with all symptoms of flacherie showed large numbers of Bacillus A. Gut slightly less alkaline than normal. The other showed no symptoms of flacherie—very few Bacillus A present. Gut normal. 1 died on 11th July 1921. Bacillus A present in large numbers. Rest died on day of spinning owing to great rise in temperature.	1 died 8th July 1921. Symptoms of flacherie, no organism. 1 died 11th July 1921. Symptoms of flacherie, micrococcus present. All control died on day of spinning owing to the temperature of the chamber suddenly going up very high (6).	Ditto
3	Ditto .	Bacillus C	Ditto	6-7-21	1 died on 8th July 1921—symptoms of flacherie—gut less alkaline than normal. Bacillus C recovered. 1 died on 12th July 1921—symptoms of flacherie—gut less alkaline than normal. Bacillus A in large numbers. Rest died on day of spinning owing to great rise in temperature.	Ditto	Ditto
4	Ditto . .	Micrococcus a	Ditto	6-7-21	All died on day of spinning owing to rise in temperature of chamber.	Ditto	Ditto

5	Ditto	. .	Bacillus A	From diseased Muga worm of March 1921. Agar slope of 2nd July 1921.	8-7-21	Ditto	Ditto	Ditto
6	Ditto	. .	Bacillus B	From moth ? flacherie of April 1921. Agar slope of 2nd July 1921.	8-7-21	Ditto	Ditto	Ditto
7	Ditto	. .	Micrococcus a	From diseased Muga worm of March 1921. Agar slope of 2nd July 1921.	8-7-21	1 died 8th July 1921 (night). Vomiting—not discoloured. Bacillus A in small numbers. Rest died on day of spinning owing to rise in temperature of chamber.	Ditto	Ditto

Experiment 27.

To see if various bad feeding methods give rise to flacherie in worms from pebrinised moth's laying.

Lot	Race	Hatching	Dates of moults	Date of spinning	How treated	Caterpillar mortality
1	Chhotapolu (54) .	28-4-21	5-5-21 10-5-21 16-5-21 24-5-21	2-6-21	Normal food	No symptoms of flacherie.
2	Do. (36) .	Do.	7-5-21 12-5-21 18-5-21 27-5-21	7-6-21	Wet fermented leaf from hatching.	12 died 29th May 1921. 2 died 30th May 1921. All showing pebrine. No symptoms of flacherie in any.
3	Do. (5) .	Do.	9-5-21 16-5-21 24-5-21 1-6-21	10-6-21	Old coarse leaf from time of hatching.	No symptoms of flacherie.
4	Do. (22) .	Do.	8-5-21 15-5-21 24-5-21 31-5-21	9-6-21	Dusty, dirty leaf from time of hatching.	Do.
5	Do. (22) .	Do.	7-5-21 12-5-21 18-5-21 27-5-21	7-6-21	As lot 2 till 3rd moult, then given dry leaf.	Do.
6	Do. (18) .	Do.	7-5-21 12-5-21 18-5-21 27-5-21	6-6-21	As lot 2 till 3rd moult, then dusty leaf.	2 died on 24th May 1921, *i.e.*, while getting wet leaf. Both pebrinised. No symptoms of flacherie.
7	Do. (8) .	Do.	9-5-21 16-5-21 24-5-21 30-5-21	9-6-21	As lot 3 but given tender leaf after 2nd moult.	No symptoms of flacherie.

Experiment 27, A.

To see if various bad feeding methods produce flacherie in the progeny of moth rejected for flacherie.

Lot	Race	How fed	Caterpillar mortality	Control
1	Nistari . . .	12 caterpillars fed on leaf dipped in 10 per cent. hydrochloric acid from 11-9-21.	nil	The rest of the laying from flacherie parent showed no signs of flacherie and gave excellent cocoons. In the May-June rearing a laying from a moth rejected for flacherie gave equally good results showing no signs of disease.
2	Do. . . .	12 caterpillars fed on wet fermented leaf.	One caterpillar was found to have its intestine coming out of its anus on 16th September 1921. It was killed when the gut contents were found to be normal. Another catterpillar with similar symptoms killed on 17th September 1921, gut contents normal.	

Experiment 28.

To see if feeding worms on acid and alkaline leaf produces flacherie.

Lot	Race	Per cent. of chemical used	How fed	Caterpillar mortality	Control
1	Chhotapolu from pebrinised moth (18) .	0·5 Citric acid .	Leaf soaked in acid fed from 16th May 1921 to 4th June 1921.	2 died on 24th May 1921. Both showed pebrine—no flacherie.	No flacherie (63).
2	Do. (22) .	1·0 Do. . .	Do. do. .	nil	Do.
3	Do. (19) .	5·0 Hydrochloric acid.	Do. do. .	1 died on 24th May 1921. Pebrine—no symptom of flacherie.	Do.
4	Nistari (12) . .	10·0 Hydrochloric acid.	Leaf soaked in acid fed from 9th June 1921 to 15th June 1921.	All died showing all external symptoms of flacherie—vomiting, diarrhœa, and discolouration. 3 died 11th June 1921. 1 died 13th June 1921. 3 died 14th June 1921. 2 died 15th June 1921. 3 dying caterpillars were killed and their gut contents cultivated. All gave almost growths of Bacillus A.	No flacherie (100).
5	Do. (12) . .	5·0 Ammonium hydrate	Leaf soaked in alkalic fed from 9th June 1921 to 18th June 1921.	2 died 15th June 1921—symptoms of flacherie as in lot No. 4. Culture made from gut gave Micrococcus a in abundance and a few colonies of Bacillus A. Rest spun showing no sign of flacherie.	Do.
6	Do. (12) . .	10·0 Ammonium hydrate.	Do. do. .	No flacherie	Do.

7	Do. (12) . .	10·0 Hydrochloric acid	Leaf soaked in acid from 30th August 1921.	No flacherie . . . . . Gut contents were of normal alkalinity.	Do.
8	Do. (6) . .	Do. do. .	Leaf soaked in acid from 6th September 1921.	Do. do. .	Do.
9	Do. (6) . .	10·0 Acetic acid .	Do. do. .	Do. do. .	Do.
10	Do. (6) . .	10·0 Nitric acid .	Do. do. .	Do. do. .	Do.

Experiment 29.

To see if flacherie is produced by feeding Muga caterpillars on organisms recovered from diseased Muga worms.

No.	Race	Organism fed	Origin of organism	When fed	Caterpillar mortality	Examination of moths	Control
1	Muga worms reared in captivity from eggs laid by moths from caterpillars brought from Assam (7).	Bacillus A. Loopfuls and smeared on leaf.	From diseased Muga worm from Assam of March 1921. Agar slope of 10th May 1921.	19th May 1921.	1 died 21st May 1921—Bacillus A in gut—some symptoms of flacherie. 1 died 1st June 1921—Bacillus A and micrococcus in gut—some symptoms of flacherie.	3 living moths—no bacteria. 2 dead moths— 1 showed no bacteria. 1 showed micrococci.	(10) 1 died 6th May 1921. Micrococci in gut. 1 died 7th June 1921. Bacillus A in gut. 8 spun cocoons and normal moths emerged.
2	Ditto (7)	Micrococcus a. Loopfuls and smeared on leaf.	Ditto	Do.	1 died 5th June 1921—Bacillus and micrococcus in gut—some flacherie symptoms. 1 died 7th June 1921—no organism—some flacherie symptoms. 1 died 8th June 1921—no organism—some flacherie symptoms.	4 living moths—no bacteria.	Ditto
3	Ditto (5)	Bacillus A. Loopfuls and smeared on leaf.	As lot 1 but agar slope of 29th April 1921.	3rd June 1921.	1 died 10th June 1921—no organisms but numerous crystals in gut—some symptoms of flacherie.	2 living moths—no bacteria. 2 dead moths—one Bacillus, other micrococci.	(6) 1 died 19th June 1921. Bacillus in gut. Rest spun—examined as moths—no bacteria.
4	Ditto (5)	Micrococcus a. Loopfuls and smeared on leaf.	Ditto	Do.	1 died 12th June 1921—Bacillus and micrococcus in gut and blood. 1 died 14th June 1921—Bacillus in blood.	1 living and 2 dead—no bacteria.	Ditto
5	Muga worms reared in captivity from eggs laid by moths sent as cocoons from Assam (12).	Bacillus A . .	From diseased Muga worm from Assam of March 1921. Agar slope of 5th June 1921.	11th June 1921.	1 died 19th June 1921—no organism. 2 died 4th July 1921—no organism but 1 caterpillar had very acid gut—brown in colour—other had symptoms of flacherie. 1 died 22nd July 1921—vomit alkaline—many micrococci and a few Bacillus in gut.	7 living moths and 1 living pupa—no bacteria.	(83) 1 died 10th July 1921. Bacillus in gut. 1 died 11th July 1921, a few micrococci in gut. Rest spun cocoons—29 living moths—no bacteria but 1 showed pebrine spores. 45 dead moths—9 showed Bacillus and 6 micrococci. 2 living and 5 dead pupæ—no bacteria.

6	Ditto	(12)	Micrococcus . .	Ditto	Do.	1 died 14th June 1921—a few Bacillus and many micrococcus in gut. 1 died 27th June 1921—numerous micrococcus in gut. 1 died 12th July 1921—no organism.	9 living moths—no bacteria.	Ditto
7	Ditto	(12)	Mixed Bacillus A and micrococcus a—chiefly Bacillus.	From diseased Muga worm of Lot one (21st May 1921). Agar slope of 5th June 1921.	Do.	2 died 20th June 1921—micrococcus in gut of one—other Bacillus in gut and blood. 1 died 29th June 1921—no organism. 1 died 29th June 1921 of injury.	5 living moths and 3 living pupæ—no bacteria.	Ditto

APPENDIX IV.

Bacillus A.

Morphology	Stout rod 3—6μ long×0·75—0·9μ wide. Rounded ends.
Motility	Rather slowly moving.
Spore formation	Abundant—central.
Gram	Positive.
Agar plate	Dirty white, rather irregular, curled, at first moist becoming rather dry, very membranous.
Agar slope	Dirty white, becoming wrinkled.
Gelatine stab.	Liquified, saccate, pellicle on surface.
Potato	Greyish, elevated, dull.
Nutrient broth	Turbid with slight ring like pellide.
Litmus milk	No change at first—slight coagulation after 1 week.
Dextrose	No gas.
Lactose	No gas.
Habitat	Diseased muga and mulberry caterpillars; healthy muga, eri and mulberry caterpillars; mulberry and castor leaf..
	Probably *Bacillus megaterium bombycis*.

Bacillus B.

Morphology	Large rod 3—6μ long×0·75μ wide, more slender than A.
Motility	Moderately to actively motile.
Spore formation	Abundant, central.
Gram	Positive.
Agar plate	Whitish, irregular to roundish, curled, very membranous.
Agar slope	Pearly white, rather spreading, with root like outgrowths, wrinkled.
Gelatine stab	Root-like outgrowths from line of inoculation, then liquifying.
Potato	Greyish, dull, raised, not very abundant at first.
Nutrient broth	Pellicle round edge later becoming dense.
Litmus milk	A little acid after 3 days.
Dextrose	No gas.
Lactose	No gas.
Habitat	Dead moths.
	Probably *Bacillus ramosus* (*mycoides*).

Bacillus C.

Morphology	Very short rods 1·5μ long×0·6μ wide.
Motility	Actively motile.
Spore formation	None.
Gram	Negative.
Agar plate	Roundish, white.
Agar slope	Moist, shining, white, filiform.
Gelatine stab	Not liquified, filiform.
Potato	Elevated, yellowish.
Nutrient broth	Turbid.
Litmus milk	Coagulated, acid.

Dextrose	Gas.
Lactose	Gas.
	Gas also given in maltose, saccharose and mannite.
Habitat	Gut of diseased caterpillar (univoltine, Kashmir).
	Probably *Bacillus coli*.

MICROCOCCUS a.

Morphology	Usually diplococcus—diam. about 0·75μ.
Gram	Positive.
Agar plate	Round, greasy, opalescent, turning dirty white and finally yellowish.
Agar slope	Filiform, greasy, opalescent turning dirty yellowish white.
Gelatine stab	No liquification, filiform, yellowish white on surface.
Potato	Raised, greasy, pale Naples yellow.
Nutrient broth	Cloudy giving ppt.
Litmus milk	Coagulated—acid after several days.
Dextrose	No gas.
Lactose	No gas.
Habitat	Diseased muga and mulberry worms; dead mulberry moths pupæ; healthy muga, eri and mulberry worms; leaf of mulberry and castor; air.
	? *Streptococcus pastorianus*, Krassilschtschik.

MICROCOCCUS b.

Morphology	Diplococcus—about 1 μ diameter.
Gram	Positive.
Agar plate	Round, opalescent becoming deep cadmium yellow, almost brown, greasy, finely granular.
Agar slope	Filiform, greasy, deep cadmium yellow.
Gelatine stab	Liquifying in form of nail with yellow ppt.
Potato	Rather flat, greasy to dry, yellow.
Nutrient broth	Turbid, yellow ppt.
Litmus milk	Coagulated—acid.
Dextrose	No gas.
Lactose	No gas.
Habitat	Diseased mulberry worms; dead mulberry moths; mulberry leaf; air.
	Probably Streptococcus a of Sasaki.
	? *Streptococcus bombycis*, Macchiati.

MICROCOCCUS c.

Morphology	Diplococcus—diam. about 0·75μ.
Gram	Positive.
Agar plate	Round, opalescent turning pale Naples yellow.
Agar slope	Filiform to echinate, greasy, whitish turning pale yellow.
Gelatine stab	No liquifaction—yellow on surface.
Potato	Scanty, colourless, greasy.
Nutrient broth	Turbid.
Litmus milk	No change.
Dextrose	No gas.
Lactose	No gas.
Habitat	Mulberry leaf and air.

MICROCOCCUS d.

Morphology	Diplococcus, at times in staphylococcus like groups—small, diam. 0·55μ.
Gram	Positive.
Agar plate	Round, greasy, sulphur yellow.
Agar slope	Filiform, greasy, sulphur yellow, slightly raised.
Gelatine stab	Liquifying in form of nail.
Potato	Raised, greasy, yellow.
Nutrient broth	Turbid, yellow ppt.
Litmus milk	Coagulated—acid after some time.
Dextrose	No gas.
Lactose	No gas.
Habitat	Gut of diseased mulberry caterpillars; gut of healthy eri and mulberry caterpillars; dead mulberry moths; mulberry leaves; air.

Probably Streptococcus o of Sasaki.
Micrococcus luteus, Lehmann and Neumann.

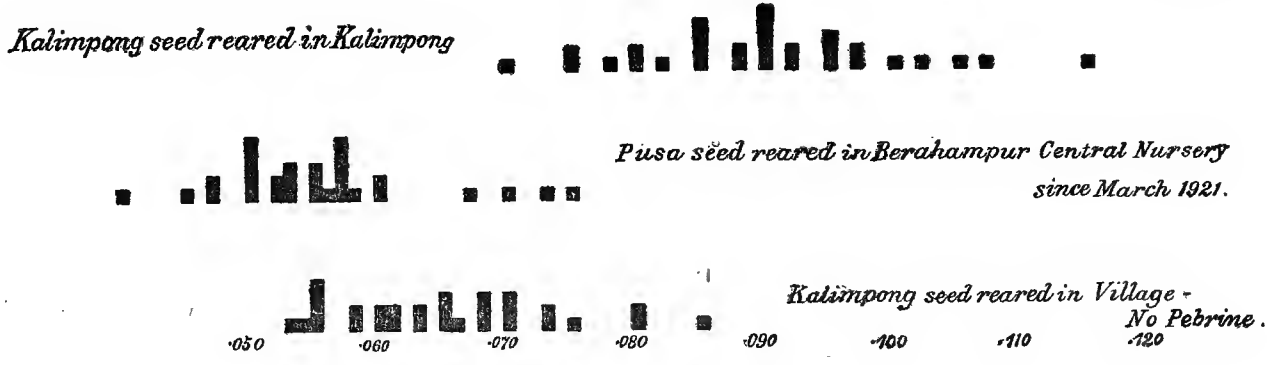
MICROCOCCUS e.

Morphology	Diplococcus—small, diam. 0·55.
Gram	Positive.
Agar plate	Round, Indian red, granular.
Agar slope	Filiform.
Gelatine stab	Liquified in form of nail.
Potato	Slight, reddish growth.
Nutrient broth	Turbid, red ppt.
Litmus milk	Coagulated—acid.
Dextrose	No gas.
Lactose	No gas.
Habitat	Dead mulberry moths; healthy mulberry caterpillars; mulberry leaves; air.

APPENDIX V.

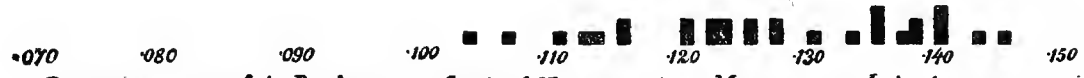
Graphs of cocoon weights in grammes.

GRAPH I.



May - June rearing

GRAPH II.

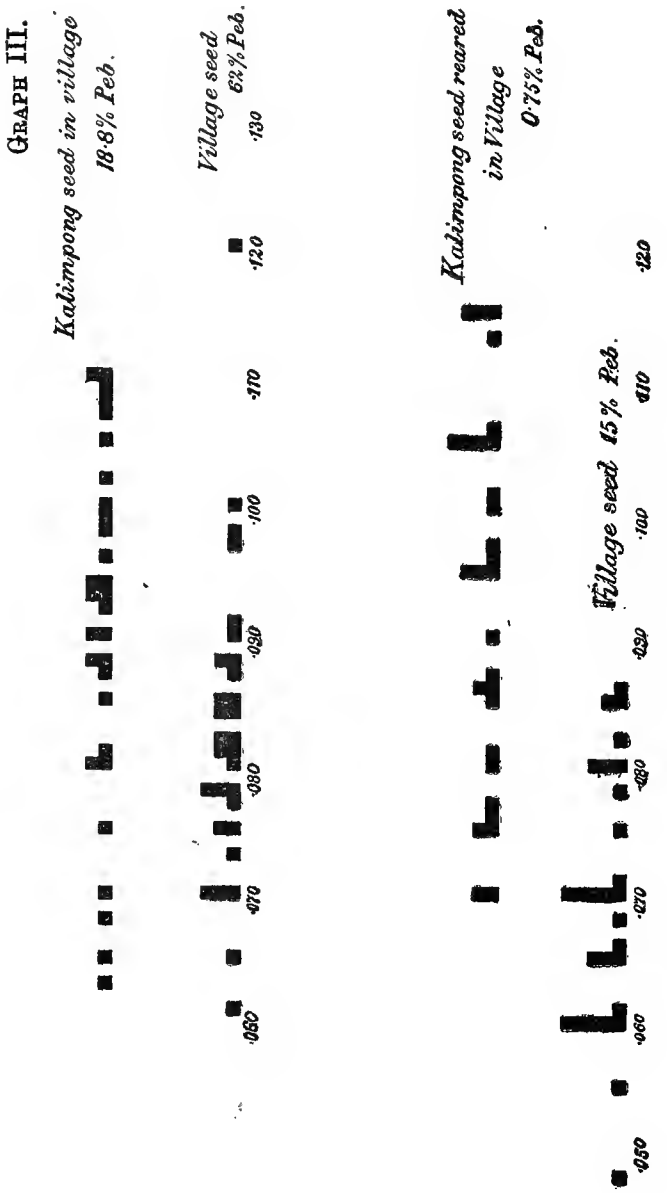


Pusa seed reared in Berhampur Central Nursery since March, 1921 July-August rearing.

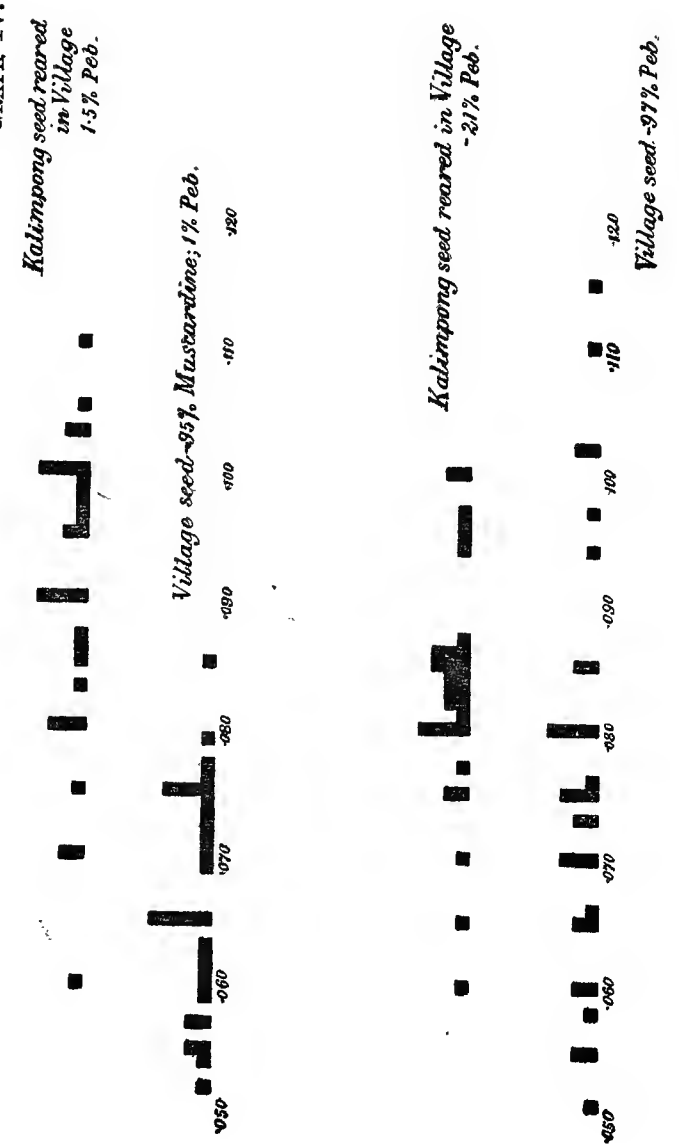
Kalimpong seed reared Berahampur Central Nursery July rearing
Original seed from Pusa-reared in Kalimpong from March-June, 1921.

Kalimpong seed reared in Kalimpong-July rearing
Original seed from Pusa reared in Kalimpong since March, 1921.

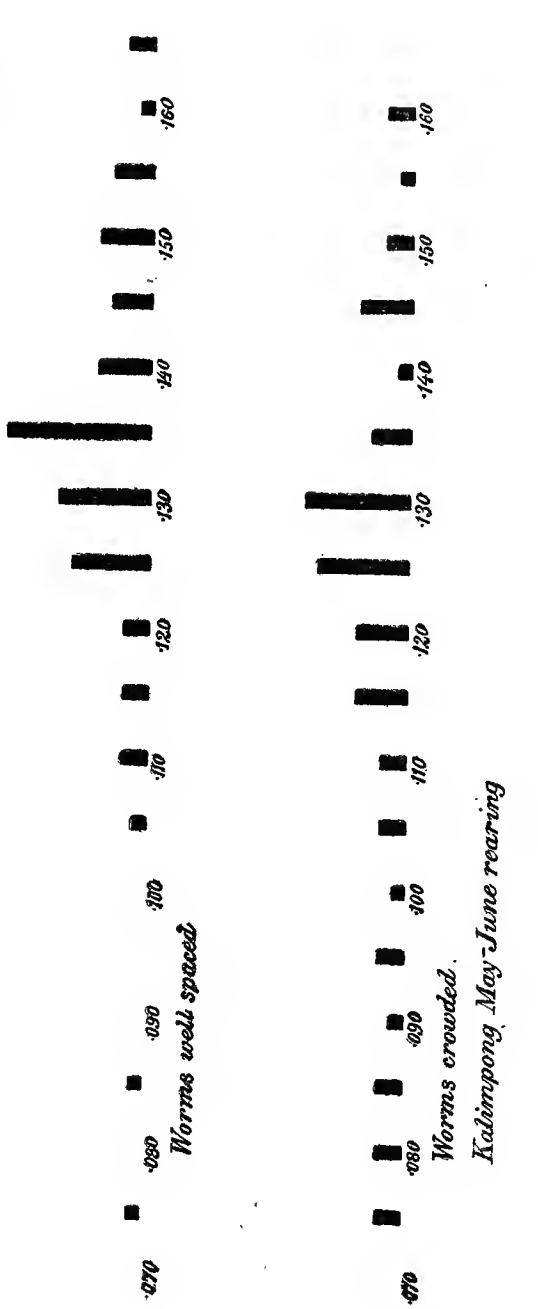
GRAPH III.


Kalimpong seed in village
18·8% Peb.
Village seed
62% Peb.
Kalimpong seed reared
in Village
0·75% Peb.
Village seed 15% Peb.
·050
·060
·070
·080
·090
·100
·110
·120
·130
·140
·150

GRAPH IV.


Kalimpong seed reared in Village 1·5% Peb.
Village seed-95% Mustardine; 1% Peb.
Kalimpong seed reared in Village -21% Peb.
Village seed.-97% Peb.
·050
·060
·070
·080
·090
·100
·110
·120

GRAPH V.


.160
.150
.140
.130
.120
.110
.100
.090
.080
.070
Worms well spaced
Worms crowded.
Kalimpong May-June rearing

BIBLIOGRAPHY.

Aoki, K. and Chigasaki, Y. (1916) . Über die Pathogenität der sog. Sottobacillen (Ishiwata) bei Seidenraupen. Bull. Imp. Sericult. Exp. St. Japan, Vol. I, No. 1, p. 97.

Banks, C. S. (1911) . . . A Manual of Philippine Silk Culture. Bureau of Science, Manilla, 1911.

Basu, B. C. (1915) . . . The Silk Industry of Assam. Shillong, 1915.

Clark, W. M. (1920) . . . The Determination of Hydrogen Ions. Baltimore, 1920.

Coutagne, G. (1902) . . . Recherches Experimentelles sur l'Heredité chez les Vers à Soie.

Geoghegan, J. (1880) . . . Silk in India. Calcutta, 1880. 2nd Ed.

Hutchinson, C. M. (1920). . . Pebrine in India. Mem. Dept. Agri. India, Bact. Ser., Vol. I, No. 8.

Jameson, A. P. and Atkins, W. R. (1921). On the Physiology of the Silkworm. Biochem. Journ., Vol. XV, No. 2, p. 209.

Krassilschtschik, J. M. (1896) . . Sur les microbes de la flacherie et de la grasserie des vers à soie. Comptes Rend. Hebd. de l'Acad. Sc., T. 123, p. 427.

Kudo, R. (1916) . . . . Contributions to the Study of ParasiticProtozoal 1. On the structure and life-history of *Nosema bombycis*, Nageli. Bull. Imp. Sericult. Exp. St. Japan, Vol. I, No. 1, p. 31.

Kudo, R. (1918) . . . . Experiments on the Extrusion of Polar Filaments of Cnidosporidean Spores. Journ. Parasit., Vol. IV, p. 141.

Lefroy, H. M. and Ghosh, C. C. (1912) Eri Silk. Mem. Dept. Agri. India, Entomol. Ser., Vol. IV, No. 1.

Lefroy, H. M. (1916) . . . Report on an Inquiry into the Silk Industry in India. Calcutta, 1916. 3 Vols.

Maillot, E. et Lambert, F. (1906) . Traité sur le ver à soie du murier et sur le murier. Montpellier, 1906.

Mukerji, N. G. (1899) . . . Handbook of Sericulture. Calcutta, 1899.

Pasteur, L. (1870) . . . . Etudes sur la maladie des vers à soie. Paris, 1870. 2 Vols.

Quatrefages, A. de . . . . Etudes sur les Maladies actuelles du ver à soie. N. D.

Sasaki, C. (1910) . . . . On the Pathology of the Jaundice (Gelbsucht) of the Silkworm. Journ. Coll. Agri. Imp. Univ. Tokyo, Vol. II, No. 2, p. 105.

Sawamura, S. (1902-03) . . . Investigation on Flacherie. Bull. Coll. Agri. Tokyo Imp. Univ., Vol. V, p. 403.

Sawamura, S. (1905) . . . On the large bacillus observed in flacherie. Bull. Coll. Agri. Tokyo Imp. Univ., Vol. VI, No. 4, p. 375.

Stempell, W. (1909) . . . Uber *Nosema bombycis*. Arch. f. Protistenk., Vol. 16.

Toyama, K. (1906-08) . . Contributions to the study of Silkworms, III On the Parasitic Fly of the domesticated Silkworms of Siam. Bull. Coll. Agri. Tokyo Imp. Univ., Vol. VII, p. 247.

Vieil, P. (1906) . . . . La Sericulture en Indo-Chine. Bull. Econom. de l'Indo-Chine., No. 57, N. S.

Watt, G. (1893) . . . . A Dictionary of the Economic Products of India, Vol. VI, Pt. III, Article—Silk. Calcutta, 1893.

White, G. F. (1919) . . . Nosema Disease. U. S. Dept. Agri. Bull. 780 (Professional paper).

The following have been seen only in abstracts :—

1913. Bull. Econom. de l'Indo-Chine, Nos. 101 and 102.

1913. Fauchere, E. Silkworm rearing in Tropical Countries. Bull. Econom. de Madagascar et dependances, Vol. 13, No. 1.

1914. Bull. Econom. de l'Indo-Chine, No. 108 (Cambodia).

Carini, A. On the partial disinfection of Mulberry leaves for Silkworms. Le Staz. sper. agrar. Italiani, Vol. XLVIII.

1917. Grangeon, F. Transmission of Richness in Silk and Hardiness in crossing Silkworms. Comptes rend. de l'Acad. d'Agric. de France, Vol. 3.

1917. Fauchere, E. Note on Sericulture in Madagascar. Comptes rend. de l'Acad. d'Agric. de France.

DESCRIPTION OF PLATES.

Plate I.

Fig. 1. Normal caterpillar for contrast with fig. 5.
„ 2. Portion of highly diseased caterpillar showing pebrine spots—especially note those on the anal horn, which frequently has a scorched appearance.
„ 3. Highly diseased moth showing distorted, crumpled wings.
„ 4. Piece of skin of caterpillar with pebrine spots, treated with caustic potash and examined under a microscope. Each spot is a little " cyst " of pebrine spores. The younger ones are smaller and lighter coloured.
„ 5. Highly diseased caterpillar showing pebrine spots.

Plate II.

Fig. 1. Portion of silk gland of highly pebrinised worm—the white patches are infected areas.
„ 2. Portion of the gut of a caterpillar fed heavily on spores and thus heavily diseased showing black spots on the gut wall—comparable with pebrine spots in the skin.
„ 3. A section through the skin of a heavily diseased worm with pebrine spots. The blue layer is the outer skin (cuticle)—the pale green is the hypodermis—the dark green is muscle—the red masses are foci of pebrine infections—the brown masses are old foci of infection which have been infiltrated with chitin.
„ 4. A younger stage in the formation of pebrine spots showing a focus of pebrine infection in the hypodermis becoming infiltrated with chitin.

Plate III.

Fig. 1. Early stage in infection of gut wall showing a few spores (oval black) near the basal membrane and a number of meronts, smaller oval bodies each dividing into 2—note the 2 nuclei in each. Semi-diagrammatic × roughly about 375.
„ 2. Section through a developing egg showing meronts of *Nosema bombycis* in the yolk. Slightly diagrammatic × roughly about 375.
„ 3. Section through the gut of a caterpillar four days after feeding it on spores, showing the extremely heavy spore formation. Note also the digestive juice buds being budded off. Semi-diagrammatic × roughly about 375.
„ 4. Spore with polar filament extruded × 2150.
„ 5. Planont just escaped from spore case × 2150.
„ 6. Planont × 1920.

Plate IV.

Diseased moth's progeny fed 4 meals a day bush mulberry. Just previous to spinning. Note great variation in size.

Plate V.

Diseased moth's progeny fed 6 meals a day bush mulberry. Just previous to spinning. Less variation in size.

Plate VI.

Diseased moth's progeny fed 6 meals a day tree mulberry. Just previous to spinning. Hardly any variation in size.

PLATE VII.

Fig. 1. Section of gut wall of caterpillar showing early stage of infection—spores and meronts in the same cells. Also digestive juice bud bursting and liberating spores into space between gut and peritrophic membrane × 364.

„ 2. Section of gut wall of caterpillar fed four days previously showing very heavy infection of spores × 300.

„ 3. Section through a developing egg showing spores in the yolk—later stage development than Plate III, fig. 2 × 300.

„ 4. Hind gut of newly hatched caterpillar from diseased moth's laying showing pebrine spores in the gut × 300.

PLATE VIII.

Fig. 1. Micrococcus a × 375.

„ 2. Micrococcus c × 375.

„ 3. Micrococcus b × 375.

„ 4. Spores of *Botrytis bassiana* (Muscardine) × 340.

„ 5. Spores of *Nosema bombycis* (Pebrine) × 375.

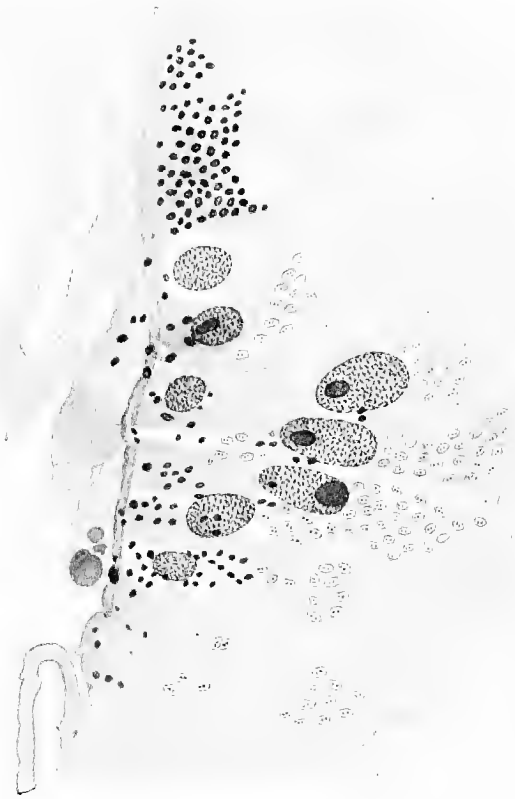
„ 6. Bacillus A—spore formation × 375.

„ 7. Bacillus A × 375.

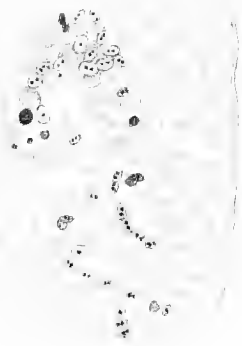
„ 8. Bacillus B × 375.

„ 9. Bacillus B—spore formation × 375.

CALCUTTA
SUPERINTENDENT GOVERNMENT PRINTING, INDIA
8, HASTINGS STREET



1



2

3

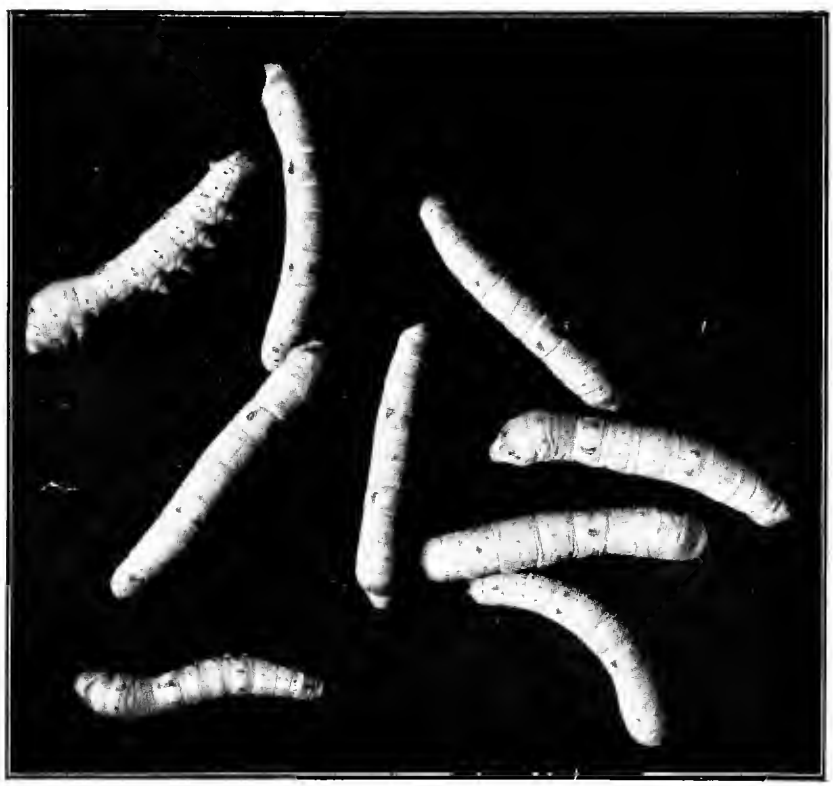
4

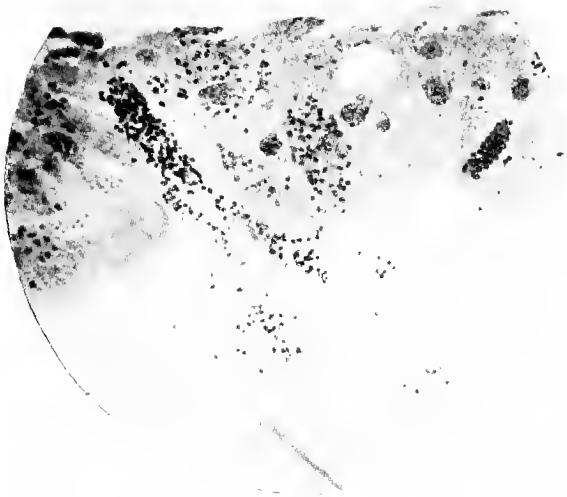
5

6

PLATE V.

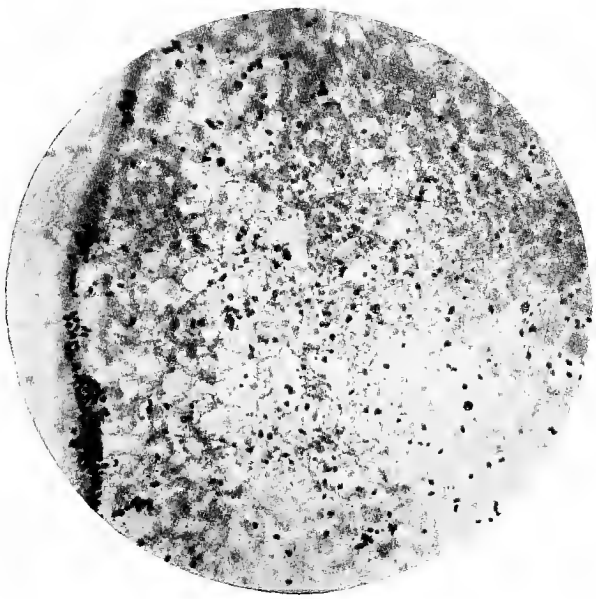
PLATE VI.



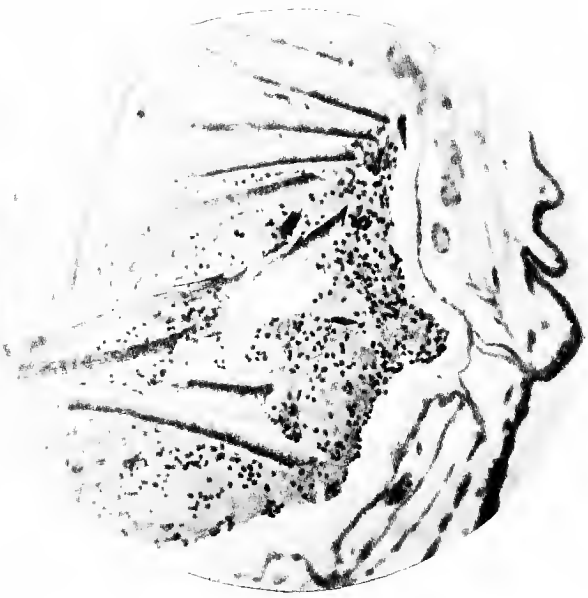


1

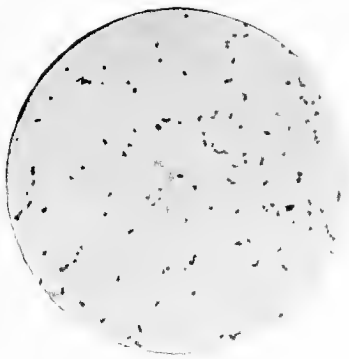
2



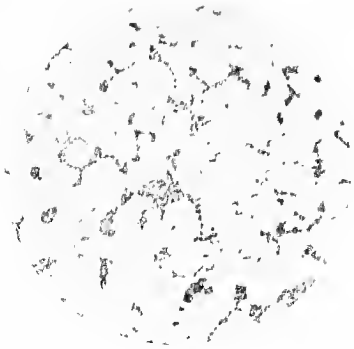
3



4



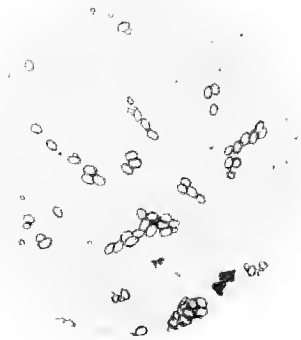
1



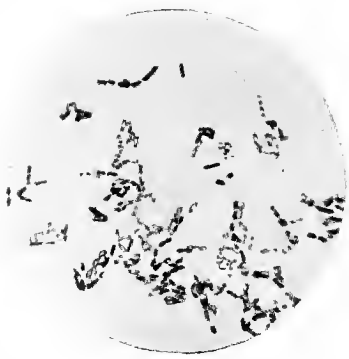
2

3

4



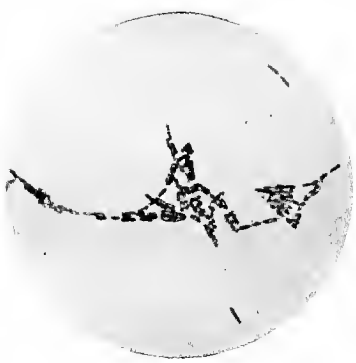
5



6

7

8



9